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METHODS FOR IDENTIFYING SUBSTANCES INTERACTING WITH PLECKSTRIN HOMOLOGY DOMAINS, AND PROTEINS CONTAINING MUTATED PLECKSTRIN HOMOLOGY DOMAINS

Abstract:

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A method for selecting or designing a compound for modulating the activity of Tandem PH domain containing Protein (TAPP), the method comprising the step of using molecular modelling means to select or design a compound that is predicted to interact with the phosphoinositide binding domain of TAPP, wherein a three-dimensional structure of at least a part of the phosphoinoisitide binding domain of the TAPP is compared with a three-dimensional structure of a compound, and a compound that is predicted to interact with the said phosphoinositide binding domain is selected. A method of selecting of designing a compound that modulates the activity of a PH domain-containing polypeptide, the method comprising the step of using molecular modelling means to select or design a compound that is predicted to interact with a PH domain, wherein a three-dimensional structure of a polycarboxylate, for example citrate, is compared with a three-dimensional structure of a coumpound, and a compound that is predicted on the basis of the structure comparison to interact with the PH domain is selected. Residues of the phosphoinositide binding site of TAPP and DAPP that are involved in determining their phosphoinositide binding specificity are identified. Polypeptides mutated at these residues are useful, for example in compound screening assays.

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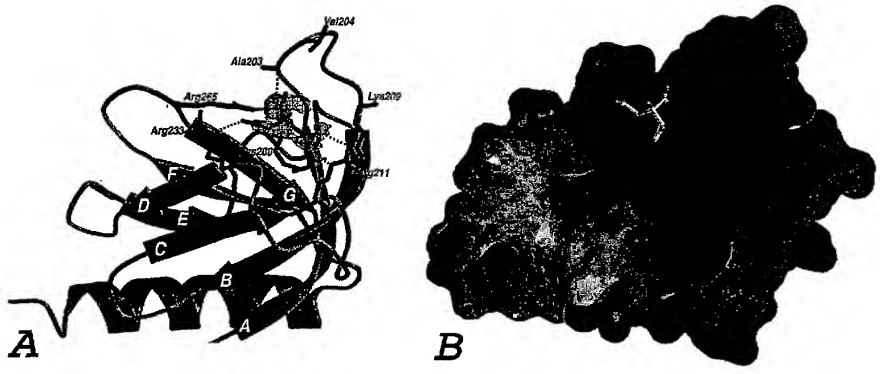

(57) Abstract: A method for selecting or designing a compound for modulating the activity of Tandem PH domain containing Protein (TAPP), the method comprising the step of using molecular modelling means to select or design a compound that is predicted to interact with the phosphoinositide binding domain of TAPP, wherein a three-dimensional structure of at least a part of the phosphoinoisitide binding domain of the TAPP is compared with a three-dimensional structure of a compound, and a compound that is predicted to interact with the said phosphoinositide binding domain is selected. A method of selecting of designing a compound that modulates the activity of a PH domain-containing polypeptide, the method comprising the step of using molecular modelling means to select or design a compound that is predicted to interact with a PH domain, wherein a three-dimensional structure of a polycarboxylate, for example citrate, is compared with a three-dimensional structure of a coumpound, and a compound that is predicted on the basis of the structure comparison to interact with the PH domain is selected. Residues of the phosphoinositide binding site of TAPP and DAPP that are involved in determining their phosphoinositide binding specificity are identified. Polypeptides mutated at these residues are useful, for example in compound screening assays.

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METHODS FOR IDENTIFYING SUBSTANCES INTERACTING WITH PLECKSTRIN HOMOLOGY DOMAINS AND PROTEINS CONTAINING MUTATED PLECKSTRIN HOMOLOGY DOMAINS

The 3-phosphoinositides phosphatidylinositol 3,4,5 trisphosphate (PtdIns(3,4,5)P_3) and PtdIns(3,4)P_2 function as cellular second messengers. Their concentrations are increased following stimulation of cells with extracellular agonists and they are thought to trigger the activation of signal transduction networks that regulate a plethora of processes including physiological responses to insulin and controlling cell survival [1, 2]. PtdIns(3,4,5)P3 is generated following growth factor/insulin-induced activation of members of the phosphoinositide 3-kinase (PI 3-kinase) family, which phosphorylate PtdIns(4,5)P_2 at the D-3 position of the inositol ring [2]. PtdIns(3,4,5)P_3 can then be converted either back to PtdIns(4,5)P_2 through the action of a 3-phosphatase termed PTEN [3] or through the action of 5-phosphatases termed SH2-containing inositol phosphatase- I (SHIP 1) or SHIP2 to PtdIns(3,4)P_2 [4]. Recent work indicates that PtdIns(3,4)P_2 can also be generated *via* a PI3-kinase independent pathway in response to agonists such as hydrogen peroxide [5] and crosslinking of platelet integrin receptors [6]. Much progress has been made on dissecting the molecular mechanisms by which PtdIns(3,4,5)P_3/PtdIns(3,4)P_2 trigger physiological processes, following the discovery that a specialised module, termed pleckstrin homology (PH) domain, is used by proteins to interact with these 3phosphoinositides [7]. Proteins that possess PH domains that can interact with PtdIns(3,4,5)P_3 include the PH domains that are found on the serine/threonine-specific protein kinases, Protein Kinase B (PKB) and the 3-Phosphoinositide Dependent Kinase-1 (PDK1) [8], the Bruton Tyrosine Kinase (BTK) family of tyrosine kinases [9], certain adaptor proteins such as Dual Adaptor of Phosphotyrosine and 3Phosphoinositides (DAPP1) [10, 11, 12, 13] and the Grb2-Associated Binder-I (Gab1) [14], as well as the ADP Ribosylation Factor (ARF), the GTPase activating protein (GAP) centaurin-α [15]

and the ARF guanine nucleotide exchange factor, GRP1 [16]. These are recruited to the plasma membrane following stimulation of cells with agonists that activate PI3-kinase, where they are brought into the vicinity of their physiological effectors and/or are activated by phosphorylation at this location of a cell (reviewed in [8]). Although there are now estimated to be ~250 proteins in the human genome that possess a PH domain, only a small proportion of these are thought to specifically interact with PtdIns(3,4,5)P_3 and or PtdIns(3,4)P_2. All of the PH domains whose structure have been determined thus far possess a conserved fold in which the N-terminal 80% of the protein forms two orthogonally arranged β-sheets, one that contains four strands (1-4), and the other of three strands (5-7), whilst the C-terminal region forms an amphipathic α-helix [7]. The structure of three PtdIns(3,4,5)P_3 binding PH domains namely, BTK [17], DAPP1 [1 8] and GRP1 [18,19] have been solved complexed to the inositol head group of PtdIns(3,4,5)P_3, inositol 1,3,4,5-tetrakisphosphate (Ins(1,3,4,5)P_4). These structures have provided insight into the molecular details of the PtdIns(3,4,5)P_3-PH domain interaction. The structures show that the inositol phosphate head group ligand binding site is formed by the side chain of the β1-β2 and β6-β7 and in some cases β3-β4 connecting loops. These loops are the most variable in length and sequence between PH domains and have been termed "the variable loops" VL1, VL2 and VL3 [20]. The basic amino acids in these loops form direct interactions with specific monoester phosphate groups of the phospho-inositol head group of PtdIns(3,4,5,)P_3 [7]. The structures elegantly explain why the PH domains of BTK [17] and GRP1 [18,19] can only interact with PtdIns(3,4,5)P_3 and not PtdIns(3,4)P_2 as the high affinity binding is mediated through interactions of the 5-phosphate on PtdIns(3,4,5)P_3 with basic residues on BTK and GRP1 PH-domains. In contrast, the DAPP1 PH domain interacts with similar affinity to both PtdIns(3,4,5)P_3 and PtdIns(3,4)P_2. It can do so because the DAPP1 PH domain,

unlike those of GRP1 and BTK, forms high affinity interactions with the 3 and 4 phosphate groups of the inositol ring and not with the 5-phosphate group that is accomodated in a largely empty space in the ligand binding pocket [18].

We recently described 2 related novel protein termed the TAndem PH domain containing Protein- I (TAPP 1) and TAPP2 because they possessed 2 sequential PH domains [21]. The N-terminal PH domain of TAPP1 and TAPP2 did not interact with any phosphoinositide tested, whilst the C-terminal PH domain bound PtdIns(3,4)P_2 with high affinity, but did not bind PtdIns(3,4,5)P_3 or any other phosphoinositide tested. TAPP1 and TAPP2 are the first proteins reported to interact specifically with PtdIns(3,4)P_2 and not with PtdIns(3,4,5)P_3 and may therefore be mediators of cellular responses that are triggered bv PtdIns(3,4)P_2.

We report the structural basis of the unusual specificity of TAPP1, a PtdIns(3,4)P_2-binding PH domain, and identify key residues that enable it to bind PtdIns(3,4)P_2 specifically. We report screening and therapeutic methods and molecules useful therein.

A first aspect of the invention provides a method for selecting or designing a compound for modulating the activity of Tandem PH domain containing Protein (TAPP), the method comprising the step of using molecular modelling means to select or design a compound that is predicted to interact with the phosphoinositide binding domain of TAPP, wherein a three-dimensional structure of at least a part of the phosphoinoisitide binding domain of the TAPP is compared with a three-dimensional structure of a compound, and a compound that is predicted to interact with the said phosphoinositide binding domain is selected.

It is preferred that the compound is for modulating the phosphoinositide binding activity of TAPP. Other activities of TAPP that may be modulated include interactions with other polypeptides, which may in turn be modulated by binding of phosphoinositides to TAPP.

Thus, the compound may be capable of affecting the intracellular location of the polypeptide; for example, it may inhibit or promote the translocation of the polypeptide to a membrane, for example the plasma membrane or golgi, vacuole, lysosome or endosome membrane. Possible association with cellular membranes of polypeptides comprising a PH domain with the required phosphoinositide binding properties are discussed further in Example 1. The compound may modulate any interaction of the polypeptide with further identical polypeptide molecules (ie self-association, for example dimerisation). A compound that is capable of modulating the ability of the polypeptide to bind to a phosphoinositide may thereby modulate the intracellular location of the polypeptide molecule and/or modulate any post-translational modification, for example phosphorylation, of the polypeptide.

It is preferred that the three-dimensional structure of at least a part of the phosphoinositide binding domain of the TAPP is a three-dimensional structure of at least a part of the phosphoinositide binding site of the TAPP and a compound that is predicted to interact with the said phosphoinositide binding site is selected. Alternatively, the compound may bind to a portion of said TAPP polypeptide that is not the binding site so as to interfere with the binding of the TAPP polypeptide to the phosphoinositide. In a still further example, the compound may bind to a portion of said polypeptide so as to decrease said polypeptide's binding activity by

an allosteric effect. This allosteric effect may be an allosteric effect that is involved in the natural regulation of the said polypeptide's activity.

It is further preferred that the three-dimensional structure of at least a part of the phosphoinositide binding site of the TAPP is a three-dimensional structure of the part of the phosphoinositide binding site of TAPP that is defined by loops β1-β2, β-3-β4, β6-β7 of full-length TAPP1 and a compound that is predicted to interact with the said part of the phosphoinositide binding site is selected.

For example, it is preferred if the portions of the structure of TAPP1 shown in Figures 1 and 2 as interacting with the phosphoinositide molecule are compared with the structure of the candidate compound.

The sequences of the loops are shown in Figure 2.

By the term Tandem Pleckstrin Homolgy domain containing protein (TAPP) is included the polypeptides termed mammalian (for example human or mouse) TAPP1 and TAPP2 identified in Dowler *et al* (2000) *Biochem J* **351**, 19-31 and in GB patent applications No 0018908.4 (filed on 3 August 2000) and 0021685.3 (filed on 5 September 2000). The term also includes fragments and fusions thereof that comprise the C-terminal PH domain, as discussed further below and in Example 1. A particularly preferred TAPP polypeptide is the C-terminal PH domain of TAPP1, optionally with an N-terminal GST tag, as described in Example 1. Further examples are considered to include fragments, variants, derivatives or fusions thereof, or fusions of fragments, variants or derivatives, that retain the phosphoinositide binding properties of full length TAPP1 or TAPP2, or

the C-terminal PH domain either thereof, as discussed further below (ie that are capable of binding to PtdIns(3,4)P_2).

By "able to bind" or "capable of binding" is meant that binding of the said polypeptide to the said phosphoinositide can be detected using a surface plasmon resonance or protein lipid overlay technique as described in Example 1 and discussed further below. By "substantially unable to bind" is meant that binding of the said polypeptide to the said phosphoinositide is not detected, or is only weakly detected using a surface plasmon resonance or protein lipid overlay technique as described in Example 1. It is preferred that the TAPP polypeptide binds to PtdIns(3,4)P_2 with at least two, preferably 3, 5, 10, 15, 20, 30 or 50-fold higher affinity than to other phosphoinositides, in particular PtdIns(3,4,5)P_3.

It is preferred that the binding of the said TAPP polypeptide to PtdIns(3,4)P_2 has an apparent K_D of less than about 2000 nM, 1000 nM or 500 nM, preferably less than about 400 or 350 nM, for example between about 350 nM and 10 nM, when measured using the method described in Example 1. It is preferred that the binding of the said polypeptide to other phosphoinositides, particularly PtdIns(3,4,5)P_3 has an apparent K_D of more than about 2000 nM, 1000 nM or 500 nM when measured using the method described in Example 1.

Methods of detecting binding of the TAPP polypeptide (for example a fragment, variant, derivative or fusion of full length TAPP, or fusion of a variant, fragment or derivative) to phospholipids, for example PtdIns(3,4)P_2, are described in Example 1, Dowler *et al* (*supra*) and GB application Nos 0018908.4 and 0021685.3 (*supra*) and include a protein-lipid overlay assay in which the lipid is spotted onto a support, for example Hybond-C extra membrane, and protein bound

to the support by virtue of interaction with the lipid is detected, for example using an antibody-based method, as well know to those skilled in the art. A surface plasmon resonance assay, for example as described in GB application Nos 0018908.4 and 0021685.3 (*supra*) or in Plant *et al* (1995) *Analyt Biochem* **226(2)**, 342-348, may alternatively be used. Methods may make use of a said polypeptide, for example comprising a PH domain, or fragment, variant, derivative or fusion thereof, or fusion of a variant, fragment or derivative that is labelled, for example with a radioactive or fluorescent label. Suitable methods may also be described in, for example, Shirai *et al* (1998) *Biochim Biophys Acta* **1402(3)**, 292-302 (use of an affinity column prepared using phosphatidylinositol analogues) and Rao *et al* (1999) *J Biol Chem* **274**, 37893-37900 (use of avidin-coated beads bound to biotinylated phosphatidylinositol analogues).

It is particularly preferred, although not essential, that the variant or fragment or derivative or fusion of the TAPP, or the fusion of the variant or fragment or derivative has at least 30% of the PtdIns(3,4)P_2 binding activity of full-length human TAPP1. It is more preferred if the variant or fragment or derivative or fusion of the said TAPP, or the fusion of the variant or fragment or derivative has at least 50%, preferably at least 70% and more preferably at least 90% of the PtdIns(3,4)P_2 binding activity of full-length human TAPP1.

In addition, it may be preferred that a variant, fragment, derivative or fusion of TAPP comprises the N-terminal of the two PH domains of TAPP. This PH domain may be capable of interacting with polypeptides, as discussed in GB application Nos 0018908.4 and 0021685.3 (*supra*). Alternatively (or in addition), it is preferred that a variant, fragment, derivative or fusion of TAPP comprises (preferably as the C-terminal three residues) the last three residues of TAPP (for

example TAPP1 or TAPP2), which conform to the minimal sequence motif (Ser/Thr-Xaa-Val/Ile) required for binding to a PDZ domain (as discussed in Example 1); and/or one or more proline rich regions found towards the C-terminus of TAPP2 (as discussed in GB application Nos 0018908.4 and 0021685.3 (*supra*), which may form a binding site for an SH3 domain).

It will be understood that it will be desirable to identify compounds that may modulate the activity of the polypeptide *in vivo*. Thus it will be understood that reagents (including any fragment, derivative, variant or fusion of the polypeptide or fusion of a variant, fragment or derivative) and conditions used in screening methods as discussed further below may be chosen such that the interactions between the said polypeptide and a phosphoinositide, for example PtdIns(3,4)P_2, or an interacting polypeptide are substantially the same as between the wild-type, preferably human polypeptide (for example TAPP1 or TAPP2) and the phosphoinositide or interacting polypeptide *in vivo*.

The sequences of human and mouse TAPP1 and TAPP2 are shown in Figures 5 and 6.

By "variants" of a polypeptide we include insertions, deletions and substitutions, either conservative or non-conservative. In particular we include variants of the polypeptide where such changes do not substantially alter the activity of TAPP, as described above.

By "conservative substitutions" is intended combinations such as Gly, Ala; Val, Ile, Leu; Asp, Glu; Asn, Gln; Ser, Thr; Lys, Arg; and Phe, Tyr.

It is particularly preferred if the TAPP variant has an amino acid sequence which has at least 65% identity with the amino acid sequence of TAPP1 or TAPP2 referred to above, more preferably at least 70%, 71%, 72%, 73% or 74%, still more preferably at least 75%, yet still more preferably at least 80%, in further preference at least 85%, in still further preference at least 90% and most preferably at least 95% or 97% identity with the amino acid sequence defined above.

It is still further preferred if the TAPP variant has an amino acid sequence which has at least 65% identity with the amino acid sequence of the C-terminal PH domain, particularly the residues forming the phosphoinositide binding site, of TAPP in the appropriate sequence referred to above, more preferably at least 70%, 71%, 72%, 73% or 74%, still more preferably at least 75%, yet still more preferably at least 80%, in further preference at least 83 or 85%, in still further preference at least 90% and most preferably at least 95% or 97% identity with the amino acid sequence defined above. It will be appreciated that the PH domain may be readily identified by a person skilled in the art, for example using sequence comparisons, for example as described in Dowler *et al* (2000) *supra* and GB Application Nos 0018908.4 and 0021685.3 (*supra*).

The percent sequence identity between two polypeptides may be determined using suitable computer programs, for example the GAP program of the University of Wisconsin Genetic Computing Group and it will be appreciated that percent identity is calculated in relation to polypeptides whose sequence has been aligned optimally.

The alignment may alternatively be carried out using the Clustal W program (Thompson *et al* (1994) *Nucl Acid Res* **22**, 4673-4680). The parameters used may be as follows:

Fast pairwise alignment parameters: K-tuple(word) size; 1, window size; 5, gap penalty; 3, number of top diagonals; 5. Scoring method: x percent.

Multiple alignment parameters: gap open penalty; 10, gap extension penalty; 0.05.

Scoring matrix: BLOSUM.

It is preferred that the TAPP is a polypeptide which consists of the amino acid sequence of the TAPP1 or TAPP2 (preferably TAPP1) sequence referred to above or naturally occurring allelic variants thereof. It is preferred that the naturally occurring allelic variants are mammalian, preferably human, but may alternatively be homologues from parasitic or pathogenic or potentially pathogenic organisms.

The TAPP may be Myc epitope-tagged or His-tagged or GST-tagged, as described in Example 1. It may be a GFP (green fluorescent protein) fusion, as known to those skilled in the art.

The method of the first aspect of the invention may further comprise the step of comparing a three-dimensional structure of at least a part of a PtdIns (3,4,5)P_3 binding domain with a three-dimensional structure of a compound, and a compound that is predicted to interact with the said phosphoinositide binding domain (or site or part thereof) of TAPP with a higher affinity than it is predicted to interact with the said PtdIns (3,4,5)P_3 binding domain is selected.

Preferably the three-dimensional structure of at least a part of the PtdIns (3,4,5)P_3 binding domain is a three-dimensional structure of at least a part of the PtdIns

(3,4,5)P_3 binding site of the PtdIns (3,4,5)P_3 binding domain and a compound that is predicted to interact with the said phosphoinositide binding domain (or site or part thereof) of TAPP with a higher affinity than it is predicted to interact with the said PtdIns (3,4,5)P_3 binding site is selected. Still more preferably, the three-dimensional structure of at least a part of the PtdIns (3,4,5)P_3 binding site is a three-dimensional structure of the part of the PtdIns (3,4,5)P_3 binding site that is defined by loops β1-β2, β-3-β4, β6-β7 of a PtdIns (3,4,5)P_3 binding domain and a compound that is predicted to interact with the said phosphoinositide binding domain (or site or part thereof) of TAPP with a higher affinity than it is predicted to interact with the said part of the PtdIns (3,4,5)P_3 binding site is selected.

Thus, it is preferred that the structures of the portion of the TAPP phosphoinositide binding site that is responsible for its specificity for PtdIns(3,4)P_2, and the portion of the PtdIns(3,4,5)P_3 binding site that is responsible for its specificity for PtdIns(3,4,5)P_3, are compared with the structure of the test compound. A compound is selected that matches this portion of the PtdIns(3,4)P_2 binding site more closely than it matches this portion of the PtdIns(3,4,5)P_3 binding site.

It is preferred that the PtdIns (3,4,5)P_3 binding domain is the PtdIns (3,4,5)P_3 binding domain of Dual Adaptor for Phosphotyrosine and Phosphoinositide (DAPP). Alternatively, the PtdIns(3,4,5)P_3 binding domain may be a mutated phosphoinositide binding domain of TAPP which is capable of binding to PtdIns(3,4,5)P_3, for example in which the residue equivalent to Ala203 is mutated, for example to a small residue, for example a glycine residue, as described in Example 1, or in which both the residue equivalent to Ala203 and the residue equivalent to Val204 are mutated, for example to a small residue, for example a

glycine residue, as described in Example 1. It is preferred that the residue equivalent to Met205 is not additionally mutated, for example to a glycine, as discussed in Example 1.

By the term DAPP is included the polypeptides termed mammalian (for example human or mouse) DAPP1 identified in Dowler *et al* (1999) *Biochem J* **342**, 7-12 (and also references 11 to 13 of Example 1) and in WO01/11042. The term also includes fragments and fusions thereof that comprise the PH domain, as discussed in Example 1, Dowler *et al* (1999; *supra*) and WO01/11042. A particularly preferred DAPP polypeptide is the PH domain of DAPP1, optionally with an N-terminal GST tag, as described in Example 1. Further examples are considered to include fragments, variants, derivatives or fusions thereof, or fusions of fragments, variants or derivatives, that retain the phosphoinositide binding properties of full length DAPP1, or the PH domain thereof, as discussed further below (ie that are capable of binding to PtdIns(3,4,5)P_3 and PtdIns(3,4)P_2).

Methods of detecting binding of the DAPP polypeptide (for example a fragment, variant, derivative or fusion of full length DAPP, or fusion of a variant, fragment or derivative) to phospholipids, for example PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3, are described in Example 1, Dowler *et al* (1999) and WO01//11042 and include techniques mentioned above in relation to TAPP.

It is particularly preferred, although not essential, that the variant or fragment or derivative or fusion of the DAPP, or the fusion of the variant or fragment or derivative has at least 30% of the PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3 binding activity of full-length human DAPP1. It is more preferred if the variant or fragment or derivative or fusion of the said DAPP, or the fusion of the variant or

fragment or derivative has at least 50%, preferably at least 70% and more preferably at least 90% of the PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3 binding activity of full-length human DAPP1.

Preferences noted above in relation to TAPP, for example in relation to sequence conservation also apply as appropriate to DAPP, as will be apparent to the person skilled in the art.

Sequences for mammalian DAPP1 are shown in Figures 7 and 8.

A further aspect of the invention provides a method for selecting or designing a compound for modulating signalling *via* PtdIns(3,4)P_2, the method comprising the step of using molecular modelling means to select or design a compound that is predicted to interact with a PtdIns(3,4)P_2-specific binding domain, wherein a three-dimensional structure of at least a part of the PtdIns(3,4)P_2 binding domain is compared with a three-dimensional structure of a compound, and a compound that is predicted to interact with the said PtdIns(3,4)P_2 binding domain is selected.

In relation to this and other aspects of the invention, the three-dimensional structures that are compared may be predicted three-dimensional structures or may be three-dimensional structures that have been determined, for example by techniques such as X-ray crystallography, as well known to those skilled in the art and as described in Example 1. The three-dimensional structures may be displayed by a computer in a two-dimensional form, for example on a computer screen. The comparison may be performed using such two-dimensional displays.

It is preferred in relation to the second aspect of the invention that the structure is of the PtdIns(3,4)P_2 binding domain of TAPP, still more preferably TAPP1. Most preferably, it is the structure reported in Example 1, for which the coordinates are shown in Figure 9.

Alternatively, the structure may be a structure of another PtdIns(3,4)P_2-specific binding domain obtained or obtainable by modelling based on the structure of the PtdIns(3,4)P_2 binding domain of TAPP and any other relevant structural information. For example, the structure may be a structure of the PH domain of DAPP mutated so that it binds PtdIns(3,4)P_2 but does not detectably bind PtdIns(3,4,5)P_3, for example mutated to replace the residue equivalent to Gly176 of full length DAPP1 with a larger residue, for example an alanine residue or other residue of similar bulk, for example serine, valine, threonine or cysteine, as described in Example 1. The structure is not a structure of a DAPP polypeptide that has the same (ie substantially the same) binding specificity as wild-type DAPP1, ie that is capable of binding to both PtdIns(3,4,5)P_3 and PtdIns(3,4)P_2.

Determined structures for DAPP1 are discussed in Example 1.

A further aspect of the invention provides method for selecting or designing a compound for modulating signalling *via* PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3 to different extents, the method comprising the step of using molecular modelling means to select or design a compound that is predicted to interact with a PtdIns(3,4)P_2 binding domain and a PtdIns(3,4,5)P_3 binding domain with different affinities, comprising the steps of (1) comparing a three-dimensional structure of at least a part of the PtdIns(3,4)P_2 binding domain with a three-dimensional structure of a compound, and (2) comparing a three-dimensional structure of at

least a part of a PtdIns $(3,4,5)P_3$ binding domain with a three-dimensional structure of the compound, and a compound that is predicted to interact with the said PtdIns$(3,4)P_2$ binding domain and the said PtdIns $(3,4,5)P_3$ binding domain with different affinities is selected.

It is preferred that the PtdIns$(3,4)P_2$ binding domain is the PtdIns$(3,4)P_2$ binding domain of TAPP. It is further preferred that the PtdIns $(3,4,5)P_3$ binding domain is the PtdIns $(3,4,5)P_3$ binding domain of DAPP. Alternatively, the PtdIns $(3,4,5)P_3$ binding domain may be a mutated phosphoinositide binding domain of TAPP which is capable of binding to PtdIns$(3,4,5)P_3$, as discussed above.

A further aspect of the invention provides a method of selecting or designing a compound that modulates the activity of a PH domain-containing polypeptide, the method comprising the step of using molecular modelling means to select or design a compound that is predicted to interact with a PH domain, wherein a three-dimensional structure of a polycarboxylate, for example citrate, is compared with a three-dimensional structure of a compound, and a compound that is predicted on the basis of the structure comparison to interact with the PH domain is selected.

As discussed in Example 1, a polycarboxylate molecule is able to bind to a PH domain, at least with low affinity. By comparing the structure of a compound with the structure of a polycarboxylate, preferably citrate, still more preferably in the conformation found in the complex with the PH domain, as described in Example 1, it may be possible to determine whether and with what affinity the compound is likely to interact with a PH domain.

Thus, a compound that can interact with the PH domain, preferably with the phosphoinositide binding site, in particular the residues considered to confer PtdIns(3,4)P_2 specificity, in a similar manner (for example similar separation and/or type of interaction ie hydrophobic or ionic, and/or similar cumulative energy of interaction) to the interacting polycarboxylate may be selected. Methods of assessing the interaction are well known to those skilled in the art.

It is preferred that the PH domain is capable of binding to a phosphoinositide and a compound that is predicted to interact with the phosphoinositide binding site of the PH domain is selected. It is further preferred that the PH domain is the phosphoinositide binding PH domain of TAPP, for example TAPP1 or TAPP2.

By "design" we include the meaning that the agent is made or modified so that it contains one or more functional groups which it is expected or known will bind all or part of the desired phosphoinositide binding domain, particularly phosphoinositide binding site, still more particularly part of a phosphoinositide binding site that is important in determining what type(s) of phosphoinositide are bound.

Skilled persons will be aware of a range of methods which can be used to design agents of the invention based on the knowledge of the PtdIns(3,4)P_2 binding site and the interaction with a polycarboxylate hereby provided.

Exemplary methods include computer aided design. In all cases, the aim of using a computer for drug design is to analyse the interactions between the drug and its receptor site and to "design" molecules that give an optimal fit. The central assumption is that a good fit results from structural and chemical complementarity

to the target receptor. The techniques provided by computational methods include computer graphics for visualisation and the methodology of theoretical chemistry. By means of quantum mechanics the structure of small molecules can be predicted to experimental accuracy. Statistical mechanics permit molecular motion and solvent effects to be incorporated.

If the molecular model of the binding site is precise enough, one can apply docking algorithms that simulate the binding of drugs to the respective receptor site. In a first step these programs create a negative image of the target site, place the putative ligands into the site and finally they evaluate the quality of the fit.

An example of the use of such design techniques is the design of potent HIV protease inhibitors (Science, 263, 1994, 380). The design was based on knowledge of the target structure.

Exemplary docking algorithm references include:-

1. B. K. Shoichet, D.L Bodian & I.D. Kuntz, "Molecular docking using shape descriptors", J. Comp. Chem. 13 (1992) 380-397.

2. I. D. Kuntz, J. M. Blaney, S J Oatley, R. L. Langridge & T.E. Ferrin, "A geometric approach to macromolecule-ligand interactions", J. Mol. Biol. 161 (1982) 269-288.

3. 2T.J.A. Ewing & I. D. Kuntz, "Critical evaluation of search algorithms for automated molecular docking and database screening", J. Comp. Chem. 18 (1997) 1175-1189.

4. E.C. Meng, B K. Shoicet & I. D. Kuntz, "Automated docking with grid-based energy evaluation", J. Comp. Chem. 13 (1992) 505-524.

5. A. R. Leach & I. D. Kuntz, "Conformational analysis of flexible ligands in macromolecular receptor sites", J. Comp. Chem 13 (1992) 730-748.

Examples of software programs for use in chemical design include MSi biosym, Tripos Sybyl, AutoDock, Software Dock, Dock and GRASP.

US Patents Nos. 5,891,643; 5,804,390; 5,698,401; and 5,989,827 relate to methods for designing compounds which bind to a specific target molecule, involving two-dimensional ^{15}N-^{1}H NMR correlation spectroscopy techniques. Such methods may be used in accordance with the present invention and the disclosure of those patents incorporated herein by reference.

The following relate to molecular modelling techniques: Blundell *et al* (1996) Stucture-based drug design *Nature* **384**, 23-26; Bohm (1996) Computational tools for structure-based ligand design *Prog Biophys Mol Biol* **66(3)**, 197-210; Cohen *et al* (1990) *J Med Chem* **33**, 883-894; Navia *et al* (1992) *Curr Opin Struct Biol* **2**, 202-210 .

The following computer programs, for example, may be useful in carrying out the method of this aspect of the invention: GRID (Goodford (1985) *J Med Chem* **28**, 849-857; available from Oxford University, Oxford, UK); MCSS (Miranker *et al* (1991) *Proteins: Structure, Function and Genetics* **11**, 29-34; available from Molecular Simulations, Burlington, MA); AUTODOCK (Goodsell *et al* (1990) *Proteins: Structure, Function and Genetics* **8**, 195-202; available from Scripps Research Institute, La Jolla, CA); DOCK (Kuntz *et al* (1982) *J Mol Biol* **161**, 269-288; available from the University of California, San Francisco, CA); LUDI (Bohm (1992) *J Comp Aid Molec Design* **6**, 61-78; available from Biosym Technologies, San Diego, CA); LEGEND (Nishibata *et al* (1991) *Tetrahedron* **47**, 8985; available from Molecular Simulations, Burlington, MA); LeapFrog (available from Tripos Associates, St Louis, MO); Gaussian 92, for example

revision C (MJ Frisch, Gaussian, Inc., Pittsburgh, PA ©1992); AMBER, version 4.0 (PA Kollman, University of California at San Francisco, ©1994); QUANTA/CHARMM (Molecular Simulations, Inc., Burlington, MA ©1994); and Insight II/Discover (Biosym Technologies Inc., San Diego, CA ©1994). Programs may be run on, for example, a Silicon Graphics™ workstation, Indigo^{2}™ or IBM RISC/6000™ workstation model 550.

By "selection" we include affinity purification techniques for identifying agents which are capable of binding to all or part of the PtdIns(3,4)P_2 binding site. Examples include affinity chromatography and phage display technologies.

The agents identified or obtained according to the above aspects of the invention (as well as the screening methods provided by further aspects of the invention, as discussed below) may be a drug-like compound or lead compound for the development of a drug-like compound. Thus, the methods may be methods for identifying a drug-like compound or lead compound for the development of a drug-like compound.

The term "drug-like compound" is well known to those skilled in the art, and may include the meaning of a compound that has characteristics that may make it suitable for use in medicine, for example as the active ingredient in a medicament. Thus, for example, a drug-like compound may be a molecule that may be synthesised by the techniques of organic chemistry, less preferably by techniques of molecular biology or biochemistry, and is preferably a small molecule, which may be of less than 5000 daltons and which may be water-soluble. A drug-like compound may additionally exhibit features of selective interaction with a

particular protein or proteins and be bioavailable and/or able to penetrate target cellular membranes, but it will be appreciated that these features are not essential.

The term "lead compound" is similarly well known to those skilled in the art, and may include the meaning that the compound, whilst not itself suitable for use as a drug (for example because it is only weakly potent against its intended target, non-selective in its action, unstable, poorly soluble, difficult to synthesise, too toxic or has poor bioavailability) may provide a starting-point for the design of other compounds that may have more desirable characteristics.

The compounds identified in the methods of the invention may themselves be useful as a drug or they may represent lead compounds for the design and synthesis of more efficacious compounds.

The selected or designed compound may be synthesised (if not already synthesised) and tested for its effect on a phosphoinositide binding domain containing polypeptide, for example TAPP or DAPP, for example its effect on the phosphoinositide binding activity. The compound may be tested in a screening method of the invention, as discussed further below.

A further aspect of the invention provides a method for identifying a compound that modulates the phosphoinositide binding activity of a PH domain containing polypeptide, comprising the step of determining the effect of the compound on the phosphoinositide binding activity of, or ability of the compound to bind to, the PH domain containing polypeptide, wherein the compound is a poly-carboxylate or variant or derivative thereof.

It is preferred that the PH domain is capable of interacting with PtdIns(3,4)P_2. It is further preferred that the PH domain-containing polypeptide is or comprises TAPP (for example TAPP1 or TAPP2) or the C-terminal pH domain of full length TAPP.

Preferably, the compound may be a variant or derivative of citrate.

When referred to herein, derivatives of -CO_2H groups include groups which are commonly derived from a carboxylic acid and/or groups that contain a central carbon atom that is at the same oxidation state as -C(O)OH. Derivatives of -CO_2H groups therefore includes groups such as:

(i) esters, e.g. those formed with an alcohol of formula R^1OH, wherein R^1 represents aryl or alkyl;

(ii) thioesters, e.g. those formed with a thiol of formula R^1SH, wherein R^1 is as hereinbefore defined; and

(iii) salts, e.g. those formed with a nitrogen-containing base such as ammonia, an alkylamine, a dialkylamine, a trialkylamine and pyridine or alkali or alkaline earth metal salts (e.g. Na, K, Cs, Mg or Ca salts).

Preferred derivatives of -CO_2H groups include those that are pharmaceutically acceptable.

It is preferred that the compound is capable of adopting a conformation that has a similar charge distribution to a citrate molecule or a PtdIns(3,4)P_2 molecule. The compound may be a phosphoinositide derivative in which one or more phosphate groups is replaced by a carboxyl group. Such a compound may be kinase-resistant.

A further aspect of the invention provides a method for identifying a compound for modulating the activity of TAPP and/or modulating signalling *via* PtdIns(3,4)P_2 or PtdIns(3,4,5)P_3, the method comprising the step of determining the effect of the compound on the activity of, or ability of the compound to bind to, (1) a mutated TAPP or mutated phosphoinositide binding domain of TAPP, mutated at a residue of loop β1-β2, β-3-β4 or β6-β7 defining at least part of the phosphoinositide binding site of TAPP, and optionally also (2) TAPP or the phosphoinositide binding domain of TAPP which is not mutated at a said residue, and selecting a compound which affects to different extents the activity of, or binds with different affinities to, the unmutated TAPP/domain and the mutated TAPP/domain.

Preferably the effect of the compound on the phosphoinositide binding activity of the mutated TAPP/domain and optionally unmutated TAPP/domain is determined. Alternatively, the effect of the compound on an activity that is modulated by phosphoinositide binding to TAPP may be measured. Examples of such activities are noted above and may include changes in conformation, cellular location and/or interaction with other polypeptide(s).

Depending upon the parameters measured, the screening method may identify compounds which inhibit binding of phosphoinositides to TAPP, and compounds which mimic binding of phosphoinositides to TAPP, as will be apparent to those skilled in the art. It will be appreciated that screens in which binding of compounds to TAPP is measured may be used in conjunction with screens in which the effect of compounds (for example compounds identified in the binding screen(s)) on one or more activities of TAPP (for example phosphoinositide binding activity) is measured.

By "mimics the effect" of the interaction of a phosphoinositide (for example PtdIns(3,4)P_2 with the TAPP is meant that the compound has a quantitative or qualitative effect on the TAPP, for example on its conformation, cellular location, or ability to interact with other polypeptide(s), that is the same as an effect of the phosphoinositide on the TAPP, for example on its conformation, cellular location, or ability to interact with other polypeptide(s).

It is preferred that the mutated TAPP/domain is capable of binding to PtdIns(3,4,5)P_3. Examples of such mutated TAPP are discussed in Example 1. It is particularly preferred that the TAPP/domain is mutated at a residue of loop β1-β2, for example at the residue equivalent to Arg212, Ala203 and/or Val204 of full length TAPP1. It is most preferred that the TAPP is mutated at the residue equivalent to Ala203 of full length TAPP1, or at the residues equivalent to Ala203 and Val204 of full length TAPP1, as discussed in Example 1.

A further aspect of the invention provides method for identifying a compound for modulating the activity of Dual Adaptor for phosphotyrosine and phosphoinositide (DAPP), and/or modulating signalling *via* PtdIns(3,4)P_2 or PtdIns(3,4,5)P_3, the method comprising the step of determining the effect of the compound on the activity of, or ability of the compound to bind to, (1) a mutated DAPP or mutated phosphoinositide binding domain of DAPP, which is capable of binding PtdIns(3,4)P_2 and is less capable of binding to PtdIns(3,4,5)P_3 than unmutated DAPP, and (2) DAPP or the phosphoinositide binding domain of DAPP which is not so mutated, and selecting a compound which affects to different extents the activity of, or binds with different affinities to, the unmutated DAPP/domain and the mutated DAPP/domain.

A further aspect of the invention provides a method for identifying a compound for modulating signalling *via* PtdIns(3,4)P_2, the method comprising the step of determining the effect of the compound on the activity of, or ability of the compound to bind to, mutated DAPP, or mutated phosphoinositide binding domain of DAPP, which is capable of binding PtdIns(3,4)P_2 and is less capable of binding to PtdIns(3,4,5)P_3 than unmutated DAPP; and selecting a compound which affects the activity of, or is capable of binding to, the said mutated DAPP/domain.

It is preferred that the DAPP/domain is mutated at the residue equivalent to Gly176 of full-length DAPP1, to a larger residue such as alanine, valine, serine, cysteine or threonine as discussed in Example 1.

Any convenient method may be used to measure the interaction of the DAPP/TAPP domain with the compound or the effect of such binding. Conveniently, the appropriate methods make use of the methods described in Example 1 for detecting and/or quantifying the interaction between a polypeptide and a phospholipid, for example a protein-lipid overlay or surface plasmon resonance method, as discussed above. It is preferred that a GST-tagged fusion polypeptide is used. Methods in which radioactively or fluorescently labelled lipids are used may also be useful.

Methods of detecting protein-protein interactions are well known to those skilled in the art. Suitable methods include yeast two-hybrid interactions, co-purification, ELISA, co-immunoprecipitation methods and cellular response assays. Cellular response assays may be carried out in a variety of cell types, for example in adipocytes or adipocyte cell lines, in a skeletal muscle cell line (such as the L6

myotubule cell line), liver cells or liver cell lines or cancer cells or cancer cell lines.

Platelets may be preferred when the polypeptide is TAPP. NIH Swiss mouse embryo cells NIH/3T3 (available from the American Type Culture Collection (ATCC) of Rockville, MD, USA (ATCC) as CRL 1658) and human embryonic kidney 293 cells (also available from the ATCC) are examples of cell lines that may be used when investigating the effect of hydrogen peroxide or other cellular stress treatment (which may lead to production of PtdIns(3,4)P_2, as noted above).

For DAPP, preferred cell types include haematopoietic cells or cell lines (for example RAW macrophages, BT cells, primary differentiated T cells). Other cell types, for example 293 cells or NIH3T3 cells may also be used.

The method (and other screening methods of the invention) may be performed *in vitro*, either in intact cells or tissues, with broken cell or tissue preparations or at least partially purified components. Alternatively, they may be performed *in vivo*. The cells, tissues or organisms in/on which the method is performed are preferably transgenic. In particular they may be transgenic for TAPP and/or DAPP, for example may express the said mutated TAPP or DAPP in place of endogenous TAPP or DAPP. They may express human TAPP or DAPP in place of endogenous TAPP or DAPP. They may express mutated DAPP in place of TAPP.

In a preferred embodiment, the interaction of the DAPP/TAPP/domain with the compound is measured using fluorescence resonance energy transfer (FRET). Alternatively, the interaction of the DAPP/TAPP/domain with the compound is measured using surface plasmon resonance.

Techniques described in GB Application Nos 0018908.4 and 0021685.3 (*supra*), adapted in view of the present teaching, may also be useful.

It will be appreciated that screening assays which are capable of high throughput operation will be particularly preferred. Examples may include cell based assays and protein-protein binding assays. An SPA-based (Scintillation Proximity Assay; Amersham International) system may be used, as well know to those skilled in the art. For example, either the polypeptide or a phosphoinositide may be immobilised on the SPA beads and the ability of the test compound to disrupt the interaction between the polypeptide and phosphoinositide may be measured. Phosphoinositides or analogues thereof may be immobilised on SPA beads, for example using methods as described in Shirai *et al* (1998) *Biochim Biophys Acta* **1402(3)**, 292-302 or in Rao *et al* (1999) *J Biol Chem* **274**, 37893-37900.

The ability of the compound to bind to the said polypeptide may be measured by measuring the ability of the compound to disrupt or prevent the interaction between the polypeptide (or variant, fragment, derivative or fusion) and the interacting phosphoinositide.

The binding constant for the binding of the compound to the relevant polypeptide may be determined. Suitable methods for detecting and/or measuring (quantifying) the binding of a compound to a polypeptide are well known to those skilled in the art and may be performed, for example using a method capable of high throughput operation, for example a chip-based method in which the compounds to be tested are immobilised in a microarray on a solid support, as known to those skilled in the art.

It will be appreciated that the screening assays of the invention are useful for identifying compounds which may be useful in the treatment of diabetes, defects of glycogen metabolism, cancer (including melanoma), inflammatory conditions, ischaemic conditions, for example stroke, thrombosis or tendency to thrombosis (for example useful as an antithrombotic agent).

The following techniques may be useful in assessing compound or phosphoinositide binding: calorimetric measurements, measurements using tritiated or other labelled phosphoinositides or headgroups, gel filtration methods (to separate bound and unbound compound or phosphoinositide), protein- lipid overlay (for example in a 384-well assay format).

The "drug-like compounds" and "lead compounds" identified in the screening assays of the invention are suitably screened in further screens to determine their potential usefulness in treating diabetes, defects of glycogen metabolism, cancer (including melanoma), inflammatory conditions, ischaemic conditions, for example stroke, or thrombosis or tendency to thrombosis. Additional screens which may be carried out include determining the effect of the compounds on blood glucose levels, tumour growth or blood clotting tendency/time, as appropriate. This may typically be done in rodents.

A further aspect of the invention provides a mutated TAPP wherein one or more residues defining the PtdIns(3,4)P_2 binding site of TAPP is mutated and the mutated TAPP is capable of binding PtdIns(3,4,5)P_3.

A further aspect of the invention provides a mutated TAPP wherein one or more of residues equivalent to residue Ala203, Val204, Met205, Thr207 of full-length human TAPP1 is mutated.

In relation to either of these aspects of the invention, it is preferred that the residue at the position equivalent to residue Ala203 is mutated to a small residue, for example Gly. It is further preferred that the residue at the position equivalent to residue Ala203 and the residue at the position equivalent to residue Val204 are both mutated to a small residue, for example Gly.

Alternatively or in addition, the residue at the position equivalent to residue Val204 is mutated to a Leu, and/or the residue at the position equivalent to residue Met205 is mutated to a Val, and/or the residue at the position equivalent to residue Thr207 is mutated to Asn. Mutation of the residue equivalent to Ala203 may be necessary for the mutated TAPP to bind to PtdIns(3,4,5)P_3. It is preferred that the residues at the positions equivalent to Ala203, Val204 and Met205 are not all mutated to Gly, as discussed in Example 1.

A further aspect of the invention provides a mutated DAPP wherein one or more residues defining the PtdIns(3,4,5)P_3 binding site of DAPP is mutated and the mutated DAPP is less capable of binding PtdIns(3,4,5)P_3 than the unmutated DAPP. It is preferred that the mutated residue is the residue equivalent to residue Gly176 of full length human DAPP1, which is preferably mutated to a larger residue, for example an alanine or another small residue, for example serine, cysteine, valine or threonine.

By "residue equivalent to" a particular residue, for example the residue equivalent to Ala203 of human TAPP1, is included the meaning that the amino acid residue occupies a position in the native two or three dimensional structure of a polypeptide corresponding to the position occupied by the said particular residue, for example Ala203, in the native two or three dimensional structure of full-length human TAPP1.

The residue equivalent to a particular residue, for example Ala203 of full-length human TAPP1, may be identified by alignment of the sequence of the polypeptide with that of full-length human TAPP1 in such a way as to maximise the match between the sequences. The alignment may be carried out by visual inspection and/or by the use of suitable computer programs, for example the GAP program of the University of Wisconsin Genetic Computing Group, which will also allow the percent identity of the polypeptides to be calculated, or using the Align program (Pearson (1994) in: Methods in Molecular Biology, Computer Analysis of Sequence Data, Part II (Griffin, AM and Griffin, HG eds) pp 365-389, Humana Press, Clifton). Thus, residues identified in this manner are also "equivalent residues".

It will be appreciated that in the case of truncated forms of human TAPP1 or DAPP1 or in forms where simple replacements of amino acids have occurred it is facile to identify the "equivalent residue".

A further aspect of the invention provides a recombinant polynucleotide suitable for expressing a mutated polypeptide of the invention. A still further aspect of the invention provides a host cell comprising a polynucleotide of the invention. In an embodiment, the host cell is capable of expressing a mutated polypeptide of the

invention in place of the unmutated polypeptide (ie in place of endogenous TAPP or DAPP).

A further aspect of the invention provides a transgenic, non-human animal comprising a host cell as defined above, in particular comprising cells which are capable of expressing a mutated polypeptide of the invention in place of the unmutated polypeptide. Techniques for generating transgenic cell lines and animals are described in Wurst & Joyner (1993) *Production of targetted embryonic stem cell clones*, in Gene Targetting (Joyner, L, Ed) ILR, Oxford.

A further aspect of the invention provides a method of making a mutated polypeptide of the invention, the method comprising culturing a host cell of the invention (preferably in cell or tissue culture) which expresses said polypeptide and isolating said polypeptide. A further aspect of the invention provides a polypeptide obtainable by this method.

Techniques involved in preparing the polypeptides, polynucleotides, host cells and transgenic animals of the invention will be well known to those skilled in the art. Exemplary techniques are described in Example 1 and references therein and also in GB application Nos 0018908.4 and 0021685.3, and WO01/11042.

A further aspect of the invention provides an antibody which binds to a mutated polypeptide of the invention but does not bind to the corresponding unmutated polypeptide. Thus, for example, the antibody may interact with an epitope comprising the amino acid sequence of TAPP flanking the residue equivalent to Ala203 or Val204 of full length TAPP1, or with an epitope comprising the amino

acid sequence of DAPP flanking the residue equivalent to Gly176 of full length DAPP1.

Antibodies reactive towards the said polypeptides may be made by methods well known in the art. In particular, the antibodies may be polyclonal or monoclonal.

Suitable monoclonal antibodies which are reactive towards the said polypeptide may be prepared by known techniques, for example those disclosed in "*Monoclonal Antibodies: A manual of techniques*", H Zola (CRC Press, 1988) and in "Monoclonal Hybridoma Antibodies: Techniques and Applications", SGR Hurrell (CRC Press, 1982).

Techniques for preparing antibodies are well known to those skilled in the art, for example as described in Harlow, ED & Lane, D "Antibodies: a laboratory manual" (1988) New York Cold Spring Harbor Laboratory.

Such antibodies may mimic binding of a phosphoinositide to the polypeptide or may alternatively or in addition act as inhibitors of phosphoinositide binding to the polyppeptide.

A further aspect of the invention provides a kit of parts useful in carrying out a screening method of the invention relating to polycarboxylates, comprising a PH domain containing polypeptide, preferably a PtdIns(3,4)P_2 binding PH domain, still more preferably TAPP or the C-terminal PH domain of full length TAPP, and at least one polycarboxylate or polycarboxylate variant or derivative compound.

A further aspect of the invention provides a kit of parts useful in carrying out other screening methods of the invention comprising (1) TAPP which is not a mutated TAPP of the invention, or DAPP which is not a mutated DAPP of the invention and (2) a mutated TAPP of the invention, or a mutated DAPP of the invention.

A further aspect of the invention provides a compound identified or identifiable by a screening or selection/design method of the invention, wherein the compound is not citrate, PtdIns(3,4)P_2, PtdIns(3,4,5), or an inositol phosphate.

The compound may be an antibody of the invention, for example an antibody capable of binding to the part of the TAPP phosphoinositide binding site that is responsible for its specificity for PtdIns(3,4)P_2, or may be a polycarboxylate molecule.

The compound may be capable of modulating the phosphoinositide binding activity of a PtdIns(3,4)P_2 binding polypeptide and a PtdIns(3,4,5)P_3 binding polypeptide and/or the PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3 binding to a phosphoinositide binding polypeptide to different extents. Thus, the compound may be capable of modulating the phosphoinositide binding activities of DAPP and TAPP to different extents, and/or may be capable of modulating the PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3 binding activities of DAPP to different extents.

It is preferred that the compound is not a phosphoinositide.

A further aspect of the invention provides a pharmaceutical composition comprising a compound identified or identifiable by a screening or selection/design method of the invention, wherein the compound is not citrate,

PtdIns(3,4)P_2, PtdIns(3,4,5), or an inositol phosphate, and a pharmaceutically acceptable excipient. A further aspect of the invention provides a said compound or composition for use in medicine.

Compounds, identifiable in the screening method, which mimic the effect of a particular phosphoinositide on a polypeptide, for example TAPP or DAPP are believed to be useful in treating diabetes and/or other conditions, as indicated above. Compounds identifiable in the screening methods of the invention that inhibit binding of a phosphoinositide to the said polypeptide are believed to be useful in treating cancer. Compounds may be used, for example, for treatment of diabetes by switching on insulin-stimulated signal transduction pathways or for the treatment of cancer by inhibiting cell proliferation or promoting apoptosis. Compounds may also be useful in the modulation or resolution of inflammation or platelet activation, as discussed above.

It will be appreciated that certain compounds found in the screening methods may be able to enhance cell proliferation in a beneficial way and may be useful, for example in the regeneration of nerves or in wound healing.

A further aspect of the invention provides the use of a said compound or composition in the manufacture of a medicament for the treatment of a patient in need of modulation of phosphoinositide binding or signalling, or a patient with an inflammatory or an ischaemic disease, cancer, diabetes, thrombosis or a defect in glycogen metabolism (or at risk of such a condition). By inflammatory disease is included immune system disorders, for example autoimmune diseases, as will be apparent to those skilled in the art.

A further aspect of the invention provides a method of treating a patient in need of modulation of phosphoinositide binding or signalling, or a patient with an inflammatory or an ischaemic disease, cancer, diabetes, thrombosis or a defect in glycogen metabolism (or at risk of such a condition), wherein the patient is administered an effective amount of a said compound or composition.

A further aspect of the invention provides a method of modulating the activity, for example phosphoinositide binding activity of a PH domain containing polypeptide (preferably TAPP or DAPP) the method comprising the step of exposing the PH domain containing polypeptide to a said compound or composition. The method may be performed *in vitro* or *in vivo*. It may be performed in cells or in cell free systems, for example using purified components.

A further aspect of the invention provides a mutated TAPP or DAPP of the invention or polynucleotide encoding said mutated TAPP or DAPP for use in medicine.

A further aspect of the invention accordingly provides a pharmaceutical composition comprising a mutated TAPP or DAPP of the invention or polynucleotide encoding said mutated TAPP or DAPP and a pharmaceutically acceptable excipient.

A further aspect of the invention provides the use of a said polypeptide or polynucleotide or composition in the manufacture of a medicament for the treatment of a patient in need of modulation of phosphoinositide binding or signalling, or a patient with an inflammatory or an ischaemic disease, cancer,

diabetes, thrombosis or a defect in glycogen metabolism (or at risk of such a condition).

A further aspect of the invention provides a method of treating a patient in need of modulation of phosphoinositide binding or signalling, or a patient with an inflammatory or an ischaemic disease, cancer, diabetes, thrombosis or a defect in glycogen metabolism (or at risk of such a condition), wherein the patient is administered an effective amount of a said polypeptide or polynucleotide or composition.

A further aspect of the invention provides the use of a mutated TAPP or DAPP of the invention, or polynucleotide encoding said mutated TAPP or DAPP in a compound screening method, for example a screening method as described above. The method may be performed *in vitro* or *in vivo*. It may be performed in cells or in cell free systems, for example using purified components.

The compound (or polypeptide or polynucleotide) may be administered to a patient (or test subject) in any suitable way, usually parenterally, for example intravenously, intraperitoneally or intravesically, in standard sterile, non-pyrogenic formulations of diluents and carriers. The compound (or polypeptide or polynucleotide) may also be administered topically. The compound (or polypeptide or polynucleotide) may also be administered in a localised manner, for example by injection.

It is preferred that the patient is in need of inhibition of PtdIns(3,4)P_2 signalling but not in need of inhibition of PtdIns(3,4,5)P_3 signalling.

Alternatively, the patient may be in need of inhibition of PtdIns(3,4,5)P_3 signalling but not in need of inhibition of PtdIns(3,4)P_2 signalling.

All documents and patent applications referred to herein are hereby incorporated by reference.

The invention is now described in further detail by reference to the following, non-limiting, Figures and Examples.

Figure legends

Figure 1. Overview TAPP1 structure + Electron density maps + GRASP surface electrostatics.

A. Ribbon drawing of TAPP1-PH_{CT}, with the seven β-strands shown as arrows with letters A-G indicating the order of the strands in the sequence. The C-terminal amphipathic α-helix is shown. Side chains of residues in the lipd head group binding pocket are shown in stick representation with black carbons, and the ordered citrate molecule is also shown. Hydrogen bonds between citrate and the protein are indicated by black dotted lines. The unbiased F_o-F_c difference map (ie prior to including a model for the citrate) is shown in orange, contoured at 2.5σ.

B. Electrostatic surface potential calculated with GRASP [27]. Darkest grey = +6 kT, mid grey = -6 kT. The citrate molecule is shown as a stick model.

Figure 2. Comparison of the DAPP1/TAPP1 PH domains

A. Structure based sequence alignment of TAPP1 residues 190-253, and DAPP1 residues 163-323. Conserved residues are highlighted in black. Arrows indicate

residues in DAPP1 that contact the inositol lipid headgroup. Underlined regions indicate the variable loops between the β-strands.

B. Stereo image oft eh TAPP1/DAPP1 ligand binding pockets. TAPP1-PH_{CT} is shown as a grey ribbon drawing, with residues lining the ligand binding pocket drawn in a sticks representation with grey carbons. The PtdIns(3,4,5)P_3 headgroup observed in the DAPP1 structure is shown with the ring in dark grey and the phosphate groups in dark and light grey. DAPP1 VL1 is shown as a string, with residues contacting the inositol head group in black. The ordered citrate molecule observed in the TAPP1-PH_{CT} structure is shown as a sticks model. Residue Ala203 in TAPP1, which is mutated to a glycine in DAPP1, is shown.

Figure 3. Phosphoinositide binding properties of wild type and VL1 mutant DAPP1 /TAPP1 PH domains. (A to K) The ability of the indicated GST fusion proteins to bind a variety of phosphoinositides was analysed using a protein-lipid overlay assay. Serial dilutions of the indicated phosphoinositides (1000 pmol, 250 pmol, 125 pmol, 63 pmol, 31 pmol, 16 pmol, 8 pmol, 4 pmol, 2 pmol and 1 pmol) were spotted onto a nitrocellulose membranes which were then incubated with the indicated wild type and mutant purified GST fusions of the isolated PH domains of the DAPP1 and TAPP1 proteins. The membranes were washed and the GST-fusion proteins bound to the membrane by virtue of their interaction with lipid were detected using a GST antibody. A representative of at least 3 separate experiments carried out is shown. An alignment of the sequences that encompass the VL1 loop in TAPP1 and DAPP1 is shown and the residues mutated are indicated. - represents an identical residue to the wild type sequence.

Figure 4. Inhibition of TAPP1 and DAPP1 binding to 3-phosphoinositides by citrate. The ability of the GST-TAPP1-$PH_{CT}$and the GST-DAPP1 isolated PH

domain to bind PtdIns(3,4,5)$_3$ or PtdIns(3,4)P_2 (or PtdIns(4,5)P_2 as a negative control) in the presence or absence of 10 mM and 100 mM trisodium citrate was analysed using the protein-lipid overlay assay described in the legend to Figure 3 except that PH domains (10 nM) were incubated for 15 min on ice in the presence of Buffer C containing 2% (by mass) BSA and either 0, 10 or 100mM trisodium citrate. Additional NaCl was added to the samples containing 0 and 10 mM trisodium citrate in order to ensure that the inonic strength was the same as the sample containing 100 mM trisodium citrate. Serial dilutions of the indicated phosphoinositides (1000pmol, 500pmol, 250pmol, 125pmol, 63pmopl, 31pmol, 16pmol, 8pmol, 4pmol, 2pmol, 1pmol and 0.5pmol) were used. Similar results were obtained in 3 separate experiments.

Figure 5: Sequences of TAPP1

Figure 6: Sequences of TAPP2

Figure 7: Sequence of human DAPP1

Figure 8: Sequence of mouse DAPP1

Figure 9: Crystal structure coordinates for TAPP1.

Example 1: Crystal structure of the phosphatidylinositol (3,4)-bisphosphate binding PH domain of TAPP1- molecular basis of lipid specificity.

Phosphatidylinositol-(3,4,5)-trisphosphate (PtdIns(3,4,5)P_3) and its immediate breakdown product PtdIns(3,4)P_2 function as second messengers in growth factor and insulin induced signalling pathways. One of the ways that these 3-

phosphoinositides are known to regulate downstream signaling events is by attracting proteins that possess specific PtdIns-binding pleckstrin-homology (PH) domains to the plasma membrane. Many of these proteins such as Protein Kinase B (PKB/Akt), 3-Phosphoinositide Dependent Protein Kinase (PDK1) and the Dual Adaptor for Phosphotyrosine and Phosphoinositide-1 (DAPP1) interact with similar affinity with both PtdIns(3,4,5)P_3 and PtdIns(3,4)P_2. Recently, a new PH domain containing protein, termed Tandem Pleckstrin Homology domain containing protein-1 (TAPP1), was described which is the first protein reported to bind PtdIns(3,4)P_2 specifically. Here we describe the crystal structure of the PtdIns(3,4)P_2 binding PH domain of TAPP1 at 1.4 Å resolution in complex with an ordered citrate molecule. Although the structure is similar to the known structure of the PH domain of DAPP1 around the D-3 and D-4 inositol phosphate binding sites, instead of a glycine residue adjacent to the D-5 inositol phosphate binding site, there is a larger alanine residue in TAPP1, which also induces a conformational change in the neighbouring residues. We show that mutation of this glycine to alanine in DAPP1 converts DAPP1 into a TAPP1-like PH domain that only interacts with PtdIns(3,4)P_2, whereas mutation in TAPP1 of the Ala to Gly permits TAPP1 to interact with moderate affinity to PtdIns(3,4,5)P_3.

The structure was solved in complex with an ordered citrate molecule, which provides a scaffold for possible PtdIns-mimetics that could have a chemotherapeutical potential.

Overall structure of the TAPP1 PH domain

The C-terminal PtdIns(3,4)P2 binding PH domain of human TAPP1 (residues 182-304, termed TAPP1 PH_{CT}) was expressed in *E. Coli*, purified and crystallised as

described in the methods section. Its structure was solved by molecular replacement and refined to 1.4 Å resolution with R=0.171, R_{free}=0.228 (Fig. 1A, Table 1; Figure 9). As expected, the overall fold of the protein is similar to previously determined PH domain structures [7]. Strands β1-β4 are arranged in an anti-parallel fashion, forming a sheet, which is approximately orthogonally stacked on another sheet formed by strands β5-β7 and β1. One open end of this β-clamp structure is covered by the C-terminal helix. Like other PH domain structures, the other open end is surrounded by loops β1 -β2, β3-β4, β6-β7, also termed VL1 (variable loop 1), VL2 and VL3, respectively. These loops form a bowl shape into which several conserved basic amino acids are clustered, forming the putative phosphoinositide binding site (Fig. 1A). Mutation of one of these residues, Arg212 to a Leu, is known to prevent TAPP1 from binding to PtdIns(3,4)P_2 [21] (Fig. 1A). Analysis of surface potential with the program GRASP [22] shows this bowl shape and its associated overall positive charge, whereas on the opposite side of the molecule a cluster of negatively charged residues can be observed (Fig. 1B). The resulting dipole is thought to help the orientation and docking of the PH domain onto the negatively charged membrane surface [7].

At the initial stages of the refinement, it became clear that the presumed PtdIns(3,4)P_2 binding pocket was occupied by an ordered small molecule (Figs. 1A). This density was not interpreted until the last stages of the refinement, when it was modeled as a citrate, which was present at a concentration of 85 mM in the crystallization mother liquor. The carboxylate moieties make several salt bridges with arginines/lysines in the pocket (Fig. 1A). Exhaustive soaking experiments of TAPP1 -PH_{CT} crystals with inositol 1,3,4-tris phosphate (the headgroup of PtdIns(3,4)P_2) were performed. Unfortunately, inspection of F_O-F_C difference maps revealed that the citrate molecule had not been replaced with the soaked

headgroup. In addition numerous, co-crystallization screens with inositol 1,3,4-tris phosphate did not yield any crystals. The TAPP1 -PH_{CT}-Citrate complex is the first structure of a PH domain bound to a non-natural ligand. Interestingly, an ordered citrate molecule has also been observed in the structure of a FYVE domain, where it also occupies the PtdIns binding site [23]. Our TAPP1 -PH_{CT}-citrate complex may provide a scaffold for the design of PtdIns-mimetics which could selectively block the recruitment of proteins carrying PH domains, thus interfering with the signal transduction cascade.

Comparison with the DAPP1 PH domain

A search for structural homologues with DALI [24] confirmed that the PH domain of DAPP1 as the most structurally similar, with an RMSD of 1.09 Å on 105 Cα atoms. A structure-based sequence alignment of the 3-phosphoinositide binding PH domains of DAPP1 and TAPP1 is shown in Fig. 2A. The sequence identity between the two PH domains is 37%. The VL1, VL2 and VL3 loops all have the same length in TAPP1 and DAPP1 and exhibit a high degree of sequence conservation. The only significant sequence differences between TAPP1 and DAPP1 in these regions are located in VL1 where Gly-Leu-Val in DAPP1 is replaced with Ala-Val-Met in TAPP1 (Fig. 2A). In order to address whether this difference in sequence between the DAPP1 and TAPP1 PH domains could account for why DAPP1 binds both PtdIns(3,4,5)P_3 and PtdIns(3,4)P_2 but TAPP1 can only bind PtdIns(3,4)P_2 we superimposed the structure of the complex of DAPP1 and inositol (1,3,4,5)P_4 with the equivalent region of TAPP1 (Fig 2B), thereby generating a model of a TAPP1-PH_{CT}-PtdIns complex (Fig. 2B). The 1, 3 and 4 phosphate groups are surrounded by identical residues (mostly arginines and lysines) in DAPP1 and TAPP1 (Fig. 2A, B). This includes Arg212, whose

mutation to a leucine is known to prevent TAPP1 from binding to PtdIns(3,4)P_2 [21] (Fig. 2B).

In contrast, the positioning of the residues surrounding the 5-phosphate in the PH domain of DAPP1 and TAPP I -PH_{CT} differ significantly. DAPP1 can bind to both PtdIns(3,4,5)P_3 and PtdIns(3,4)P_2 because the 5-phosphate can be accommodated in the ligand binding pocket. Furthermore, in comparison to the 1, 3 and 4 phosphate groups (see introduction), the 5-phosphate in the DAPP1_PH-PtdIns(3,4,5)P_3 complex has relatively weak interactions with the protein, through two hydrogen bonds with the backbone nitrogens of residues Leu177 and Val178 (Fig. 2B). In the TAPP! -PH_{CT} structure, there is no room for the 5-phosphate to bind. If a 5-phosphate is positioned in TAPP1-PH_{CT} in the same place it occupies in the DAPP1-PH-inositol(1,3,4,5)P_4 complex, severe clashes are introduced with Ala203 (equivalent to Gly176 in DAPP1), with a distance of 1.4 Å between Ala2O3-C β and an oxygen on the 5-phosphate (Fig. 2B). Another notable steric clash is Val204-Cα with an oxygen on the 5-phosphate (2.2 Å). In addition the two phosphate-protein hydrogen bonds seen in the DAPP1_PH-inositol(1,3,4,5)P_4 complex can no longer be formed if inositol(1,3,4,5)P_4 is modeled into the TAPP1 phosphoinositide binding site.

It is possible that it is not only the steric effects caused by introduction of a Cβ atom at position 203 which could prevent TAPP1 interacting with a phosphoinositide bearing a 5-phosphate group. Inspection of the backbone conformation reveals that Glyl76 in DAPP1 lies at ϕ=-83°, ψ=-83° in the Ramachandran plot, whereas for the equivalent Ala203 in TAPP1 ϕ=-79°, ψ=-31°. According to the PROCHECK definition [25], the backbone conformation at residue 176 in DAPP1 is near the edge of an "additional allowed" region in the

Ramachandran plot, whereas residue 203 in TAPP1 is near the center of a highly populated allowed region (right-handed α helical). Thus, it is possible that by the introduction of a Cβ beyond the glycine backbone, the VL1 loop is forced in a different conformation to relieve the strain introduced in the backbone. This would cause the observed shifts of up to 4.1 Å in Cα positions towards the site of PtdIns binding, resulting in the additional unfavourable changes in interaction with a phosphoinositide bearing a 5-phosphate.

Mutagenesis of residues located in the VL1 loop of TAPP1 and DAPP1 PH domains

The results presented above indicate that mutation of Gly176 in DAPP1 to an Ala, i.e. the residue found at the equivalent position in TAPP1, may inhibit the binding of DAPP1 to PtdIns(3,4,5)P_3 but not affect its interaction with PtdIns(3,4)P_2. To investigate this, we employed the protein-lipid overlay assay [26, 21], in which serial dilutions of phosphoinositides were spotted on to a nitrocellulose membrane and incubated with the isolated PH domain of wild type DAPP1 and mutant DAPP1 [G176A]. The membranes were then washed and immunoblotted with a GST antibody to detect GST fusion proteins bound to the membrane by virtue of their interaction with lipid. In Fig. 3A we demonstrate that as reported previously [10, 11] wild type DAPP1 interacts with both PtdIns(3,4,5)P_3 and PtdIns(3,4)P_2 but the mutant DAPP1 [G176A] as predicted only bound to PtdIns(3,4)P_2 (with similar apparent affinity as wild type DAPP1) but did not detectably bind to PtdIns(3,4,5)P_3 (Fig 3 B).

We next mutated either individually or in combination the residues Ala-Val-Met in the VL1 loop of TAPP1 to residues present in the equivalent region of DAPP1 (Gly-Leu-Val) to determine whether we could generate a TAPP1 mutant that could

interact with PtdIns(3,4,5)P_3 as well as PtdIns(3,4)P_2. The TAPP1 mutant in which Ala203 (the residues equivalent to G176 in DAPP1) was changed to a Gly residue TAPP1[A203G] showed significantly interaction with PtdIns(3,4,5)P_3 compared to the wild type TAPP1, but it should be noted still bound to PtdIns(3,4)P_2 with a higher affinity (Fig. 3C, D). In contrast, the TAPP1[V204L] only interacted with PtdIns(3,4)P_2 but with reduced affinity compared to that of type TAPP1 (Fig 3E). The TAPP1[M205V] mutant also exclusively bound to PtdIns(3,4)P_2 with similar apparent affinity as wild type TAPP1. A mutant of TAPP1 in which the 3 residues in the VL1 loop of TAPP1 (e.g. AVM) were altered to those found in DAPP1 (e.g. GLV) was able to interact with PtdIns(3,4,5)P_3 to a similar extent as the TAPP1 [A203G] mutant (compare Figs 3D and 3G). We then mutated either individually or in combination further residues in the Ala-Val-Met motif of TAPP1 to Gly residues. The TAPP1[V204G] interacted only with PtdIns(3,4)P_2 like wild type TAPP1 (Fig 3H). In contrast, the TAPP1[A203G, V204G] mutant interacted with PtdIns(3,4,5)P_3 with significantly higher affinity than the TAPP1[A203G] mutant and remarkably even possessed higher affinity for PtdIns(3,4,5)P_3 than PtdIns(3,4)P_2 (Fig 3I). We also generated a mutant TAPP1 in which all 3 residues in the Ala-Val-Met motif were changed to Gly, but this mutant failed to interact with either PtdIns(3,4)P_2 or PtdIns(3,4,5)P_3 (Fig 3J). 2 Residues C-terminal to the AVM sequence in TAPP1 there is a Thr residue (Thr207) whereas in DAPP1 this residue is an Asn (Asn180). However, this difference does not appear to contribute to the differences of lipid binding specificity of TAPP1 and DAPP1 because a mutant TAPP1 in which this Thr residue is changed to Asn still interacted specifically with PtdIns(3,4)P_2 (Fig 3K).

As the TAPP1 -PH_{CT} structure obtained had an ordered citrate molecule bound in the lipid binding pocket (Figs. 1A, 2B) and we were unable to obtain crystals of TAPP1 bound to inositol(1,3,4)P_3, we verified whether citrate could inhibit the binding of TAPP1-PH_{CT} and the isolated PH domain of DAPP1 to 3-phosphoinositides. We observed moderate inhibition at 10 mM citrate, but at a concentration of 100 mM citrate (similar to that present in the crystallisation mother liquor) the ability of TAPP1-PH_{CT} to interact with PtdIns(3,4)P_2 and the DAPP1 PH domain to bind PtdIns(3,4,5)P_3 and PtdIns(3,4)P_2 was significantly reduced (Fig 4). As the apparent Kd of TAPP1 for PtdIns(3,4)P_2 and DAPP1 for PtdIns(3,4,5)P_3/PtdIns(3,4)P_2 are estimated to be sub-micromolar [10, 21], citrate must therefore interact with these PH domains with > 10^5 lower affinity than the natural phosphoinositide ligand(s) of these PH domains. Together with the structure, these data suggest that poly-carboxylates may be attractive molecules for the design of PH-domain-specific PtdIns mimetics.

Materials & Methods

Materials

All the phosphoinositides used in this study were dipalmitoyl derivatives obtained from Cell Signals. Hybond-C extra, the pGEX4T-1 vector, Enhanced Chemiluminescence reagent, Thrombin protease and Glutathione-Sepharose were from Amersham Pharmacia Biotech; Protease Inhibitor tablets were from Roche; Benzamidine-Agarose and monoclonal anti-Glutathione-S-Transferase (GST) antibody was from Sigma; Anti-goat anti mouse-horse radish peroxidase conjugated secondary antibody was from Pierce; VivaSpin concentrators were from Viva Sciences.

General methods and Buffers

Restriction enzyme digests, DNA ligations, Polymerase Chain Reaction cloning and site directed mutagenesis were performed using standard protocols. All DNA constructs were verified by DNA sequencing. Buffer A: 50 mM Tris-HCl pH 7.5, 1 mM EGTA, 1 mM EDTA, 1 mM sodium orthovanadate, 10 mM sodium-β-glycerophosphate, 50 mM sodium fluoride, 5 mM dithiothreitol and "complete" proteinase inhibitor cocktail (one tablet per 25 ml). Buffer B: 50mM Tris/HCl pH 7.5, 0.1 mM EGTA, 0.2 M NaCl and 5 mM dithiothreitol. Buffer C: 50 mM MES/NaOH pH6.0, 150 mM NaCl and 0.1 % (by mass) Tween-20.

Preparation of PH domain expression constructs

The PtdIns(3,41)P_2 binding PH domain of TAPP1 (TAPP1-PH_{CT}) was amplified by PCR using the Hi-fidelity PCR system with the full length TAPP1 cDNA [21] as the template, and the 5' primer GATCCATGTTTACTCCTAAACCACCTCAAGATAG and the 3'primer GGATCCTCAGGGATGCTCAGAAGACGCAGATCT. This amplified a DNA encoding residues 182 to 304 of human TAPP1 with a stop codon immediately after position 304 which is equivalent in length to the DAPP1 PH domain fragment that was crystallised previously [18]. This fragment was subcloned into the *Bam*HI restriction site of the *E. coli* expression vector pGEX4T-1. The resultant construct encodes for the bacterial expression of TAPP1 -PH_{CT} with an N-terminal GST tag. This construct was used for both crystallisation and for generating the mutants of TAPP1-PH_{CT} employed in the lipid binding experiments shown in Fig. 3. The pGEX4T-1 construct encoding for the expression of the GST fusion of the isolated PH domain of human DAPP1 (residues 154 to 273) was described previously [10] and was used to generate the pGEX4T-1 construct expressing the isolated GST-DAPP1 [G176A] mutant PH domain.

Expression and purification of TAPP1-PH_{CT} for crystallisation

E. coli BL21 cells transformed with the pGEX4T-1 vector encoding the expression of GST-TAPP1-PH_{CT} were grown at 37 °C in 2 litres of Luria Broth with 50 mg/ml carbenicillin until OD_{600} reached 0.7. The expression of GST-TAPP1-PH_{CT} was induced by the addition of 250 mM isopropyl-β-D-thiogalactopyranoside, and the bacteria were then grown for a further 18 hours at 27 °C. The cells were harvested by centrifugation at 3500 x g for 15 min, then lysed by resuspension in 100 ml of Buffer A containing DNAse and lysozyme and passing through a French Press. The resulting solution was centrifuged at 13000 g for 30 min to remove residual debris, briefly sonicated and passed through a 0.45 μM filter. The supernatant was incubated for one hour at 4 °C in 4 ml of Glutathione-Sepharose previously equilibrated in Buffer A, and then the beads washed 6 times with 5 column volumes of Buffer B. The TAPP1 PH domain was then separated from the GST-tag by incubating the Glutathione Sepharose beads conjugated to GST-TAPP1-PH_{CT} in a 1:1 volume of resin to Buffer B with thrombin 100 U/ml at 4°C overnight. The resin was centrifuged, washed twice with 2 volumes of Buffer B and the combined supernatants containing TAPP1-PH_{CT} were applied to a 0.2ml Benzamidine Agarose column to remove the thrombin. The eluate from this column was subsequently applied to a 1ml Glutathione Sepharose column equilibrated in Buffer B to remove trace contamination of GST. The yield obtained was approximately 8 mg of TAPP1 -PH_{CT} domain per 2 litres of *E. coli* culture. TAPP1-PH_{CT} was analysed by SDS-polyacrylamide gel electrophoresis followed by Coomasie Blue staining of the gel and found to be essentially homogenous. It was analysed by electrospray mass spectroscopy, revealing a

major single species of molecular mass of 14138.39, close to the predicted mass of 14162.24 for the TAPP1-PH_{CT} fragment.

Crystallisation and data collection

The TAPP1-PH_{CT} protein was concentrated to a final concentration of 14.5 mg/ml (as determined by a Bradford assay) using a VivaSpin concentrator. Hanging drops were formed by mixing 1µl of protein solution with I µl of a mother liquor solution containing 0.085 M sodium citrate, 25.5% PEG 4000, 15% glycerol, and 0.17 M ammonium acetate. The drop was then equilibrated by vapour diffusion against a reservoir containing mother liquor. Crystals were grown at 20°C and appeared after 1 day, growing to 0.3mm x 0.15mm x 0.1mm over 6 days. Crystals were frozen straight from the drop without additional cryo-protection. Data were collected to 1.4 Å at ESRF beamline ID14-EH4, using an ADSC Q4 CCD detector, at a wavelength of 1.0Å. The temperature of crystal was maintained at 100K using a nitrogen cryostream. Data were processed using the HKL package [27], statistics are shown in Table 1.

Structure determination and refinement

The structure of the TAPP1 PH domain was solved by molecular replacement with AMoRe [28], using the DAPP1 structure [18] as a search model. A single solution was obtained with an R-factor of 0.501 and a correlation coefficient of 0.392. The resulting model phases were used as input for warpNtrace [29] which was able to build 92 out of the 123 possible residues. Iterative model building in O [30] together with refinement in CNS reduced the R-factor to 0.213 (R_{free}=0.231). Refinement was then continued with SHELX97 [31], incorporating anisotropic B-

factors, and as a last step riding hydrogens, resulting in the final model with an R-factor to 0.175 (R_{free}=0.221) (Table 1).

Protein-lipid overlay

The wild type and mutant PH domains of TAPP1 and DAPP1 used for the lipid binding experiments were expressed in *E. coli* and purified as described before [21]. Lipid binding studies were carried out using the protein-lipid overlay assay described previously [10, 21]. Briefly, lyophilised lipids were reconstituted in 1:1 mixture of chloroform:methanol to a concentration of 1 mM. The stock solution was serially diluted by 2-fold in a mixture chloroform:methanol:water (1:2:0.8) and 1ml of this solution, corresponding to between 1000 and 1 pmole of phosphoinositide, was spotted onto HybondC-extra nitrocellulose membrane, which was allowed to dry at room temperature for 1 hr. Membranes were blocked for 1 hr at room temperature in Buffer C containing 2% (by mass) bovine serum albumin (BSA) and then were incubated in Buffer C containing 2% (by mass) BSA and 10 nM of the indicated purified wild type or mutant GST-fusion PH domains of DAPP1 and TAPP1. After incubation overnight at 4°C with gentle rocking, the membranes were washed in 5 times over 40 min in Buffer C, then incubated with 1:2000 dilution of a monoclonal anti-GST antibody in Buffer C containing 2% by mass BSA for 1 h at room temperature. Membranes were washed a further 5 times over 40 min in Buffer C and then incubated with 1:5000 dilution of goat anti-mouse secondary antibody conjugated to horse Radish Peroxidase In Buffer C containing 2% (by mass) BSA for 1 hr. The membranes were washed 8 times over 1 hour and detection of GST fusion proteins bound to the membrane was achieved by enhanced chemiluminescence.

Table 1

Details of data collection and refinement. All measured data were included in the refinement.

Space group	$C222_1$
Unit cell	a= 65.02
	b= 102.80
	c= 41.47
Resolution, last bin ()	30-1.4 (1.45-1.4)
Unique reflections	27661
Redundancy	4.0 (3.1)
Completeness (%)	98.9 (97.7)
R_{merge}	0.058 (0.115)
I/σI	21.7 (4.2)
R_{cryst}	0.171
R_{free}	0.228
Number of atoms	874 protein
	134 water
Wilson B-factor (^{2})	20.1
< B > protein (^{2})	26.9
< B > (water)	44.1

R.M.S. deviations from ideal geometry

Bond lengths	0.010Å
Bond angles	1.98°
Main chain B-factors	2.93 Å^2

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CLAIMS

1. A method for selecting or designing a compound for modulating the activity of Tandem PH domain containing Protein (TAPP), the method comprising the step of using molecular modelling means to select or design a compound that is predicted to interact with the phosphoinositide binding domain of TAPP, wherein a three-dimensional structure of at least a part of the phosphoinoisitide binding domain of the TAPP is compared with a three-dimensional structure of a compound, and a compound that is predicted to interact with the said phosphoinositide binding domain is selected.

2. The method of claim 1 wherein the compound is for modulating the phosphoinositide binding activity of TAPP.

3. The method of claim 1 or 2 wherein the three-dimensional structure of at least a part of the phosphoinositide binding domain of the TAPP is a three-dimensional structure of at least a part of the phosphoinositide binding site of the TAPP and a compound that is predicted to interact with the said phosphoinositide binding site is selected.

4. The method of claim 3 wherein the three-dimensional structure of at least a part of the phosphoinositide binding site of the TAPP is a three-dimensional structure of the part of the phosphoinositide binding site of TAPP that is defined by loops β1-β2, β-3-β4, β6-β7 of full-length TAPP1 and a compound that is predicted to interact with the said part of the phosphoinositide binding site is selected.

5. The method of any of the preceding claims further comprising the step of comparing a three-dimensional structure of at least a part of a PtdIns (3,4,5)P_3 binding domain with a three-dimensional structure of a compound, and a compound that is predicted to interact with the said phosphoinositide binding domain (or site or part thereof) of TAPP with a higher affinity than it is predicted to interact with the said PtdIns (3,4,5)P_3 binding domain is selected.

6. The method of claim 5 wherein the three-dimensional structure of at least a part of the PtdIns (3,4,5)P_3 binding domain is a three-dimensional structure of at least a part of the PtdIns (3,4,5)P_3 binding site of the PtdIns (3,4,5)P_3 binding domain and a compound that is predicted to interact with the said phosphoinositide binding domain (or site or part thereof) of TAPP with a higher affinity than it is predicted to interact with the said PtdIns (3,4,5)P_3 binding site is selected.

7. The method of claim 6 wherein the three-dimensional structure of at least a part of the PtdIns (3,4,5)P_3 binding site is a three-dimensional structure of the part of the PtdIns (3,4,5)P_3 binding site that is defined by loops β1-β2, β-3-β4, β6-β7 of a PtdIns (3,4,5)P_3 binding domain and a compound that is predicted to interact with the said phosphoinositide binding domain (or site or part thereof) of TAPP with a higher affinity than it is predicted to interact with the said part of the PtdIns (3,4,5)P_3 binding site is selected.

8. A method for selecting or designing a compound for modulating signalling *via* PtdIns(3,4)P_2, the method comprising the step of using molecular modelling means to select or design a compound that is predicted to interact with a PtdIns(3,4)P_2 binding domain, wherein a three-dimensional structure of at least a part of the PtdIns(3,4)P_2 binding domain is compared with a three-dimensional

structure of a compound, and a compound that is predicted to interact with the said PtdIns(3,4)P_2 binding domain is selected.

9. A method for selecting or designing a compound for modulating signalling *via* PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3 to different extents, the method comprising the step of using molecular modelling means to select or design a compound that is predicted to interact with a PtdIns(3,4)P_2 binding domain and a PtdIns(3,4,5)P_3 binding domain with different affinities, comprising the steps of (1) comparing a three-dimensional structure of at least a part of the PtdIns(3,4)P_2 binding domain with a three-dimensional structure of a compound, and (2) comparing a three-dimensional structure of at least a part of a PtdIns (3,4,5)P_3 binding domain with a three-dimensional structure of the compound, and a compound that is predicted to interact with the said PtdIns(3,4)P_2 binding domain and the said PtdIns (3,4,5)P_3 binding domain with different affinities is selected.

10. The method of claim 9 wherein the PtdIns(3,4)P_2 binding domain is the PtdIns(3,4)P_2 binding domain of TAPP.

11. The method of any one of claims 5 to 7 or 9 wherein the PtdIns (3,4,5)P_3 binding domain is the PtdIns (3,4,5)P_3 binding domain of DAPP.

12. The method of any one of claims 5 to 7 or 9 wherein the PtdIns (3,4,5)P_3 binding domain is a mutated phosphoinositide binding domain of TAPP which is capable of binding to PtdIns(3,4,5)P_3.

13. A method of selecting or designing a compound that modulates the activity of a PH domain-containing polypeptide, the method comprising the step of using

molecular modelling means to select or design a compound that is predicted to interact with a PH domain, wherein a three-dimensional structure of a polycarboxylate, for example citrate, is compared with a three-dimensional structure of a compound, and a compound that is predicted on the basis of the structure comparison to interact with the PH domain is selected.

14. The method of claim 13 wherein the PH domain is capable of binding to a phosphoinositide and a compound that is predicted to interact with the phosphoinositide binding site of the PH domain is selected.

15. A method for identifying a compound that modulates the phosphoinositide binding activity of a PH domain containing polypeptide, comprising the step of determining the effect of the compound on the phosphoinositide binding activity of, or ability of the compound to bind to, the PH domain containing polypeptide, wherein the compound is a poly-carboxylate or variant thereof.

16. The method of any one of claims 13 to 15 wherein the PH domain is capable of interacting with PtdIns(3,4)P_2.

17. The method of any one of claims 13 to 16 wherein the PH domain-containing polypeptide is or comprises TAPP or the C-terminal PH domain of full length TAPP.

18. A method for identifying a compound for modulating the activity of TAPP and/or modulating signalling *via* PtdIns(3,4)P_2 or PtdIns(3,4,5)P_3, the method comprising the step of determining the effect of the compound on the activity of, or ability of the compound to bind to, (1) a mutated TAPP or mutated

phosphoinositide binding domain of TAPP, mutated at a residue of loop β1-β2, β-3-β4 or β6-β7 defining at least part of the phosphoinositide binding site of TAPP, and optionally also (2) TAPP or the phosphoinositide binding domain of TAPP which is not mutated at a said residue, and selecting a compound which affects to different extents the activity of, or binds with different affinities to, the unmutated TAPP/domain and the mutated TAPP/domain.

19. The method of claim 18 wherein the effect of the compound on the phosphoinositide binding activity of the TAPP/domain and mutated TAPP/domain is determined.

20. The method of claim 18 or 19 wherein the mutated TAPP/domain is capable of binding to PtdIns(3,4,5)P_3.

21. The method of claim 18, 19 or 20 wherein the TAPP/domain is mutated at a residue of loop β1-β2.

22. The method of any one of claims 18 to 21 wherein the TAPP/domain is mutated at the residue equivalent to Arg212, Ala203 and/or Val204 of full length TAPP1.

23. A method for identifying a compound for modulating the activity of Dual Adaptor for phosphotyrosine and phosphoinositide (DAPP), and/or modulating signalling *via* PtdIns(3,4)P_2 or PtdIns(3,4,5)P_3, the method comprising the step of determining the effect of the compound on the activity of, or ability of the compound to bind to, (1) a mutated DAPP or mutated phosphoinositide binding domain of DAPP, which is capable of binding PtdIns(3,4)P_2 and is less capable of

binding to PtdIns(3,4,5)P_3 than unmutated DAPP, and (2) DAPP or the phosphoinositide binding domain of DAPP which is not so mutated, and selecting a compound which affects to different extents the activity of, or affinity of binding to, the unmutated DAPP/domain and the mutated DAPP/domain.

24. A method for identifying a compound for modulating signalling *via* PtdIns(3,4)P_2, the method comprising the step of determining the effect of the compound on the activity of, or ability of the compound to bind to, mutated DAPP, or mutated phosphoinositide binding domain of DAPP, which is capable of binding PtdIns(3,4)P_2 and is less capable of binding to PtdIns(3,4,5)P_3 than unmutated DAPP; and selecting a compound which affects the activity of, or is capable of binding to, the said mutated DAPP/domain.

25. The method of claim 23 or 24 wherein the DAPP/domain is mutated at the residue equivalent to Gly176 of full-length DAPP1.

26. The method of any of the preceding claims wherein the interaction of the DAPP/TAPP/domain with the compound is measured using fluorescence resonance energy transfer (FRET).

27. The method of any of the preceding claims wherein the interaction of the DAPP/TAPP/domain with the compound is measured using surface plasmon resonance.

28. A mutated TAPP wherein one or more residues defining the PtdIns(3,4)P_2 binding site of TAPP is mutated and the mutated TAPP is capable of binding PtdIns(3,4,5)P_3.

29. A mutated TAPP wherein a mutated residue(s) is the residue equivalent to residue Ala203, Val204, Met205 or Thr207 of full-length human TAPP1.

30. The mutated TAPP of claim 28 or 29 wherein the residue at the position equivalent to residue Ala203 is mutated to a Gly and optionally the residue at the position equivalent to residue Val204 is mutated to a Gly.

31. The mutated TAPP of claim 29 or 30 wherein the residue at the position equivalent to residue Val204 is mutated to a Leu, and/or the residue at the position equivalent to residueMet205 is mutated to a Val, and/or the residue at the position equivalent to residue Thr207 is mutated to Asn.

32. A mutated DAPP wherein one or more residues defining the PtdIns(3,4,5)P_3 binding site of DAPP is mutated and the mutated DAPP is less capable of binding PtdIns(3,4,5)P_3 than the unmutated DAPP.

33. The mutated DAPP of claim 32 wherein a mutated residue is the residue equivalent to residue Gly176 of full length human DAPP1.

34. A recombinant polynucleotide encoding or suitable for expressing a mutated polypeptide according to any of claims 28 to 33.

35. A host cell comprising a polynucleotide according to claim 34.

36. The host cell of claim 35 wherein the said host cell is capable of expressing a mutated polypeptide according to any one of claims 28 to 33 in place of the unmutated polypeptide.

37. A transgenic, non-human animal comprising a host cell according to claim 35 or 36.

38. A method of making a mutated polypeptide according to any one of claims 28 to 33, the method comprising culturing a host cell according to claim 35 or 36 which expresses said polypeptide and isolating said polypeptide.

39. A polypeptide obtainable by the method of claim 38.

40. An antibody which binds to a mutated polypeptide as defined in any one of claims 28 to 33 but does not bind to the equivalent unmutated polypeptide.

41. A kit of parts useful in carrying out a method according to any one of claims 15 to 17, comprising a PH domain containing polypeptide as defined in any one of claims 15 to 17 and at least one polycarboxylate or polycarboxylate variant compound.

42. A kit of parts useful in carrying out a method according to any one of claims 18 to 27, comprising (1) TAPP which is not a mutated TAPP according to any one of claims 28 to 31, or DAPP which is not a mutated DAPP according to claim 32 or 33 and (2) a mutated TAPP according to any one of claims 28 to 31, or a mutated DAPP according to any one of claims 32 to 33.

43. A compound identified or identifiable by the method of any one of claims 1 to 27, wherein the compound is not citrate, PtdIns(3,4)P_2, PtdIns(3,4,5), or an inositol phosphate.

44. The compound of claim 43 wherein the compound is capable of modulating the phosphoinositide binding activity of a PtdIns(3,4)P_2 binding polypeptide and a PtdIns(3,4,5)P_3 binding polypeptide and/or the PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3 binding to a phosphoinositide binding polypeptide to different extents.

45. The compound of any one of claims 43 or 44 wherein the compound is not a phosphoinositide.

46. A pharmaceutical composition comprising a compound according to any one of claims 43 to 45 and a pharmaceutically acceptable excipient.

47. A compound according to any one of claims 43 to 45 or composition according to claim 46 for use in medicine.

48. Use of a compound or composition as defined in claim 47 in the manufacture of a medicament for the treatment of a patient in need of modulation of phosphoinositide binding or signalling.

49. A method of modulating the phosphoinositide binding activity of a PH domain containing polypeptide the method comprising the step of exposing the PH domain containing polypeptide to a compound or composition as defined in claim 46.

50. A mutated TAPP or DAPP according to any one of claims 28 to 33 or polynucleotide according to claim 34 for use in medicine.

51. A pharmaceutical composition comprising a mutated TAPP or DAPP according to any one of claims 28 to 33 or polynucleotide according to claim 34 and a pharmaceutically acceptable excipient.

52. The use of a composition or polypeptide or polynucleotide as defined in claim 50 or 51 in the manufacture of a medicament for the treatment of a patient in need of modulation of phosphoinositide binding or signalling.

53. The use of claim 52 or 48 wherein the patient is in need of inhibition of PtdIns(3,4)P_2 signalling but not in need of inhibition of PtdIns(3,4,5)P_3 signalling.

54. The use of claim 52 or 48 wherein the patient is in need of inhibition of PtdIns(3,4,5)P_3 signalling but not in need of inhibition of PtdIns(3,4)P_2 signalling.

55. The use of any one of claims 48 or 52 to 54 wherein the patient has an inflammatory or an ischaemic disease, cancer (particularly melanoma), diabetes, thrombosis or a defect in glycogen metabolism (or at risk of such a condition).

Figure 1

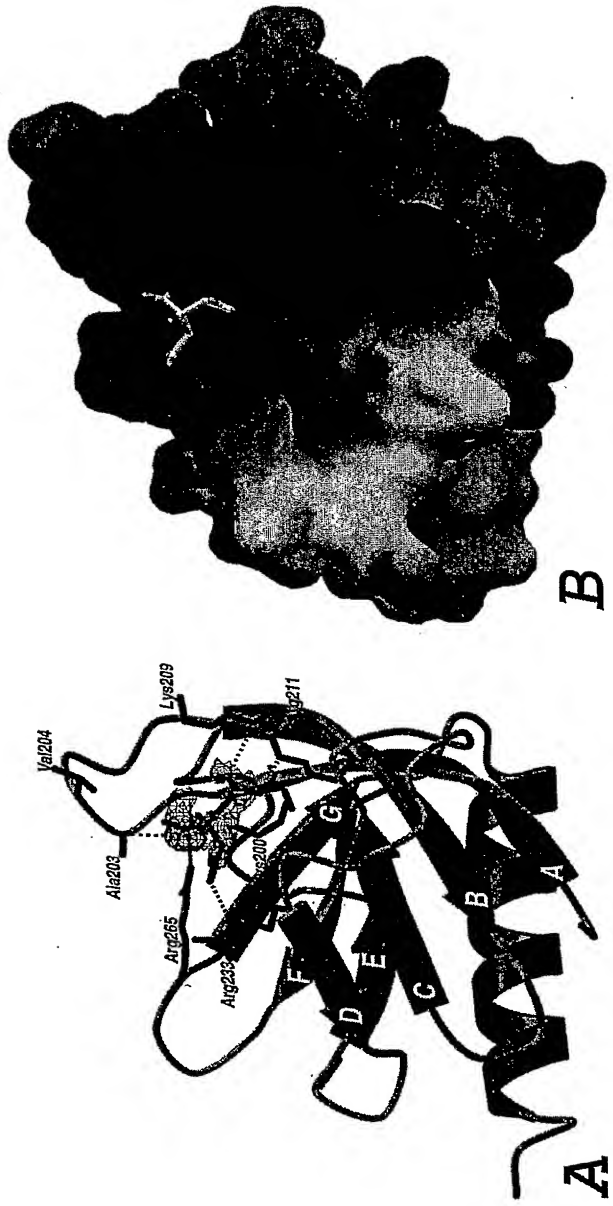


Figure 2: page 1

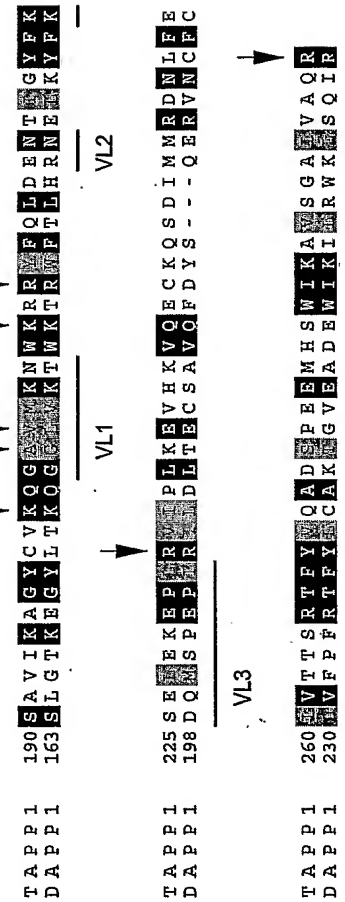
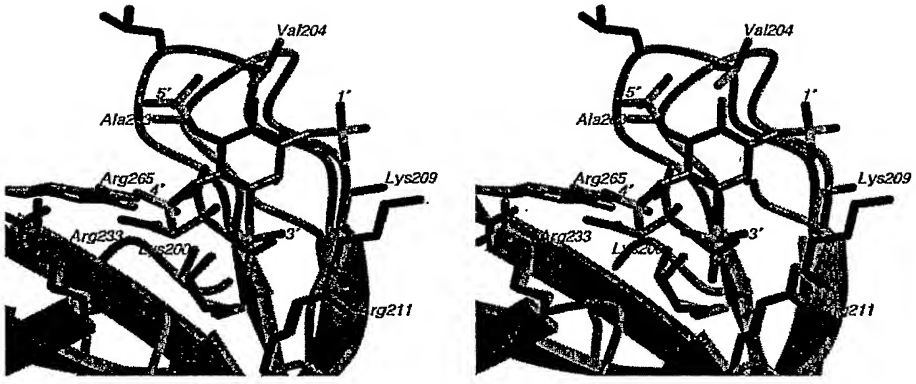
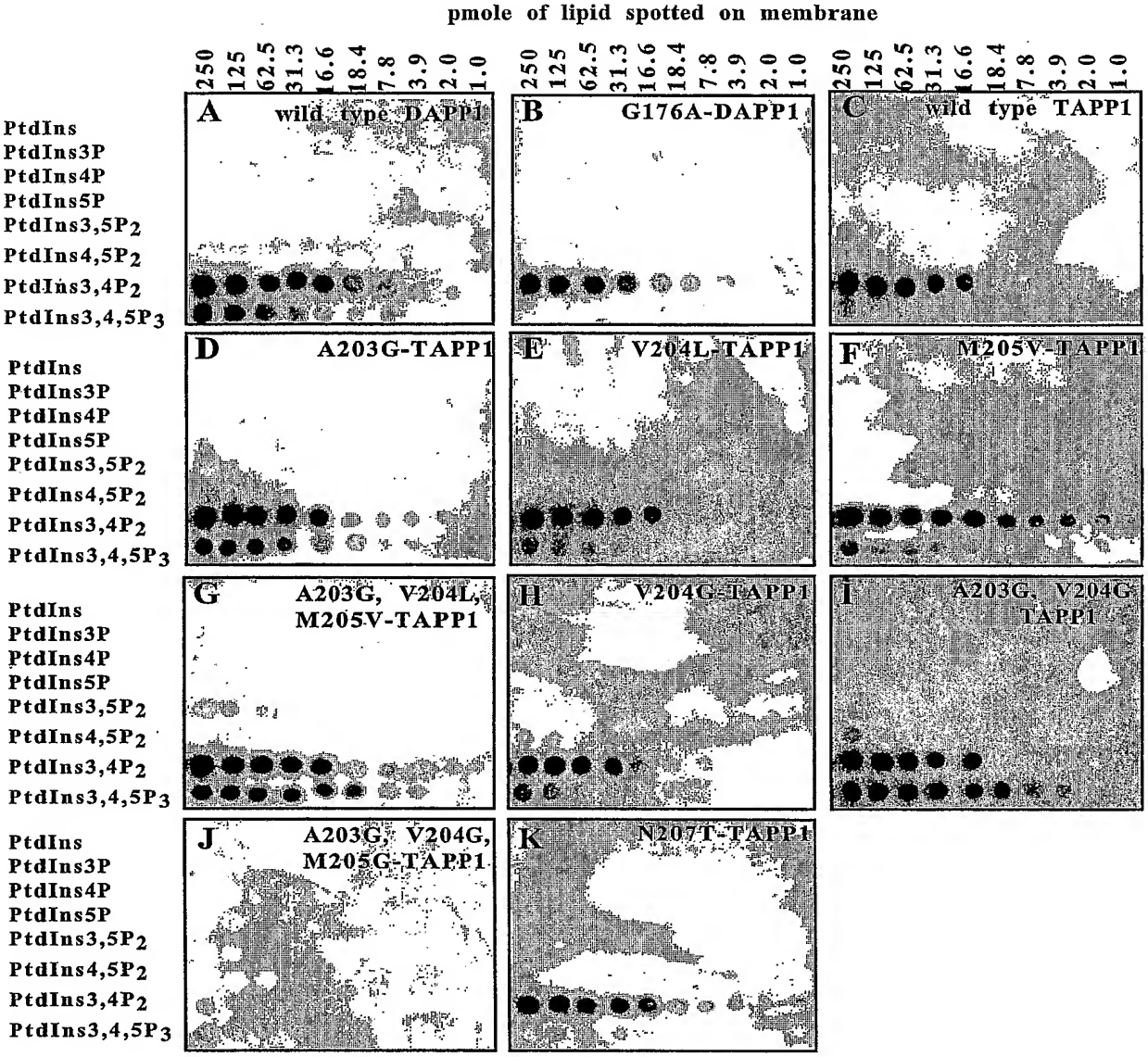


Figure 2: page 2





DAPP1 wild type	GGLVKT
DAPP1 G176A	-A----
TAPP1 wild type	GAVMKN
TAPP1 A203G	-G----
TAPP1 V204L	--L---
TAPP1 M205V	---V--
TAPP1 A203G,V204L,M205V	-GLV--
TAPP1 V204G	--G---
TAPP1 A203G,V204G	-GG---
TAPP1 A203G,V204G,M205G	-GGG--
TAPP1 N207T	-----T

Figure 3

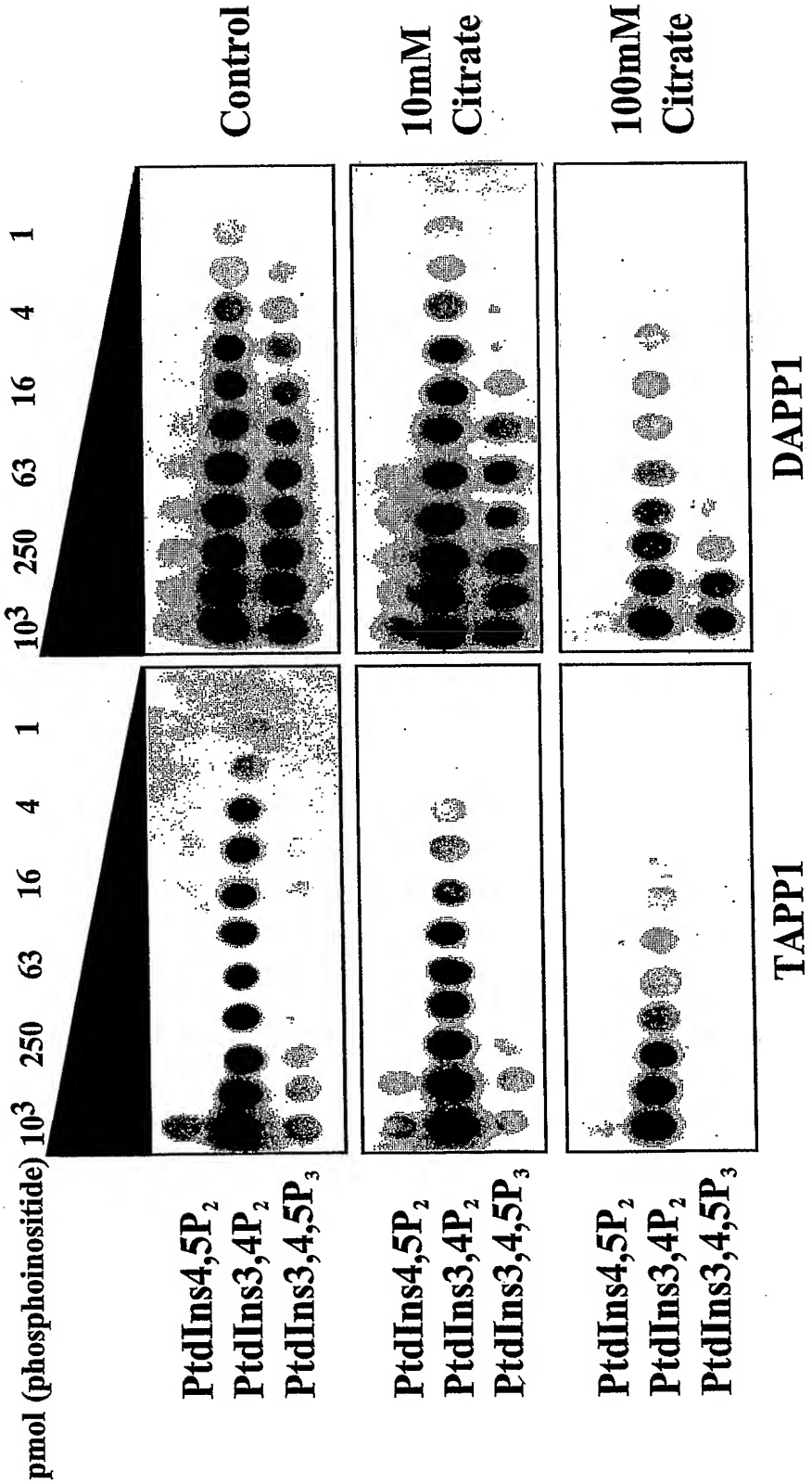


Figure 4

MPYVDRQNRICGFLDIEENENSGKFLRRYFILDTREDSFVWYMDNPQNLP
SGSSRVGAIKLTYISKVSDATKLRPKAEFCFVMNAGMRKYFLQANDQQDL
VEWVNVLNKAIKITVPKQSDSQPNSDNLSRHGECGKKQVSYRTDIVGGVP
IITPTQKEEVNECGESIDRNNLKRSQSHLPYFTPKPPQDSAVIKAGYCVK
QGAVMKNWKRRYFQLDENTIGYFKSELEKEPLRVIPLKEVHKVQECKQSD
IMMRDNLFEIVTTSRTFYVQADSPEEMHSWIKAVSGAIVAQRGPGRSASS
EHPPGPSESKHAFRPTNAAATSHSTASRSNSLVSTFTMEKRGFYESLAK
VKPGNFKVQTVSPREPASKVTEQALLRPQSKNGPQEKDCDLVDLDDASLP
VSDV

(human TAPP1 amino acid sequence)

MPYVDRQNRICGFLDIEENENSGKFLRRYFILDTREDSFVWYMDNPQnnn
nnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnn
nMNAGMRKYFLQANDQQDLVEWVNVLNKAIKITVPKQSDSQPASDSLSRQ
GDCGKKQVSYRTDIVGGVPIITPTQKEEVNECGESLDRNNLKRSQSHLPY
FAPKPPSDSAVIKAGYCVKQGAVMKNWKRRYFQLDENTIGYFKSELEKEP
LRVIPLKEVHKVQECKQSDIMMRDNLFEIVTTSRTFYVQADSPEEMHSWI
KAVSGAIVAQRGPGRSSSSnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnn

(partial mouse TAPP1 amino acid sequence; the run of n's indicates a gap of unknown length)

Figure 5

Figure 6

RGEREARRVWQADPEIPGARRTRRPEGRPRPM*RAPPEPRPLHGGG*CEQ
SPGMPYVDRQNRICGFLDIEEHENSGKFLRRYFILDTQANCLLWYMDNPQ
NLAMGAGAVGALQLTYISKVSIATPKQKPKTPFCFVINALSQRYFLQAND
QKDMKDWVEALNQASKITVPKGGGLPMTTEVLKSLAAPPALEKKPQVAYK
TEIIGGVVVHTPISQNGGDGQEGSEPGSHTILRRSQSYIPTSGCRASTGP
PLIKSGYCVKQGNVRKSWKRRFFALDDFTICYFKCEQDREPLRTIFFKDV
LKTHECLVKSGDLLMRDNLFEIITSSRTFYVQADSPEDMHSWIKEIGAAV
QALKCHP

(partial human TAPP2 amino acid sequence)

MPYVDRQNRICGFLDIEDNENSGKFLRRYFILDTQANCLLWYMDNPQNLA
VGAGAVGSLQLTYISKVSIATPKQKPKTPFCFVINALSQRYFLQANDQKD
LKDWVEALNQASKITVPKAGTVPLATEVLKNLTAPPTLEKKPQVAYKTEI
IGGVVVQTPISQNGGDGQEGCEPGTHAFLRRSQSYIPTSGCRPSTGPPLI
KSGYCVKQGNVRKSWKRRFFALDDFTICYFKCEQDREPLRTIPLKDVLKT
HECLVKSGDLLMRDNLFEIITTSRTFYVQADSPEDMHSWIEGIGAAVQAL
KCHPREPSFSRSISLTRPGSSTLTSAPNSILSRRRPPAEEKRGLCKAPSV
ASSWQPWTPVPQAEEKPLSVEHAPEDSLFMPNPGESTATGVLASSRVRHR
SEPQHPKEKPFVFNLDDENIRTSDV

(mouse TAPP2 amino acid sequence)

MGRAELLEGKMSTQDPSDLWSRSDGEAELLQDLGWYHGNLTRHAAEALLLSNG

CDGSYLLRDSNETGLYSLSVRAKDSVKHFHVEYTGYSFKFGFNEFSSLKDFV

KHFANQPLIGSETGTLMVLKHPYPRKVEEPSIYESVRVHTAMQTGRTEDDLVP

TAPSLGTKEGYLTKQGGLVKTWKTRWFTLHRNELKYFKDQMSPEPIRILDLTE

CSAVQFDYSQERVNCFCLVFPFRTFYLCAKTGVEADEWIKILRWKLSQIRKQL

NQGEGTIRSRSFIFK

Human DAPP1

Figure 7

MGRAELLGGNMSTQDPSELWGRADGGTDLLQDLGWYHGNLTRHAAEALLLSNG

RDGSYLLRDSNEQTGLYSLSVRAKDSVKHFHVEYTGYSFKFGFNEYSSLKDFV

KHFANQPLIGSETGTLMVLKHPYPREVEEPCIYESVRVHTAMQTGRTENDLVP

TAPSLGTKEGYLTKQGGLVKTWKTRWFTLQRNELKYFKDQMSPEPIRILDLTE

CSAVQFDYSQERVNCFCLVFPFRTFYLCAKTGVEADEWIKILRWKLSKIRKQL

DQGEDTVRSRSFIFK

Mouse DAPP1

Figure 8

Figure 9: page 1

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HEADER    DUMMY FOR WHATIF
COMPND    DUMMY
SOURCE    G. VRIENDS IMAGINATION
AUTHOR    G. VRIEND
CRYST1 1000.000 1000.000 1000.000  90.00  90.00  90.00 P 1
SCALE1       .001000  0.000000  0.000000        0.00000
SCALE2     0.000000  0.001000  0.000000        0.00000
SCALE3     0.000000  0.000000  0.001000        0.00000
ATOM      1  N   SER   190       5.581   5.884  16.623  1.00 56.83
ATOM      2  CA  SER   190       5.697   6.493  15.301  1.00 54.92
ATOM      3  C   SER   190       5.771   8.009  15.423  1.00 52.39
ATOM      4  O   SER   190       5.486   8.783  14.504  1.00 53.89
ATOM      5  CB  SER   190       4.528   6.106  14.385  1.00 57.18
ATOM      6  OG  SER   190       4.901   5.108  13.445  1.00 62.31
ATOM      7  N   ALA   191       6.174   8.480  16.612  1.00 48.06
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ATOM      9  C   ALA   191       7.592  10.385  15.955  1.00 40.31
ATOM     10  O   ALA   191       8.428   9.611  15.479  1.00 38.28
ATOM     11  CB  ALA   191       6.377  10.388  18.161  1.00 48.96
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ATOM     17  CG1 VAL   192       9.530  14.581  14.248  1.00 29.40
ATOM     18  CG2 VAL   192       7.116  13.800  14.023  1.00 33.79
ATOM     19  N   ILE   193      11.100  11.834  15.218  1.00 25.18
ATOM     20  CA  ILE   193      12.425  11.753  15.824  1.00 23.78
ATOM     21  C   ILE   193      13.184  13.072  15.651  1.00 22.70
ATOM     22  O   ILE   193      13.854  13.528  16.574  1.00 25.07
ATOM     23  CB  ILE   193      13.214  10.599  15.186  1.00 24.11
ATOM     24  CG1 ILE   193      12.575   9.229  15.488  1.00 27.33
ATOM     25  CG2 ILE   193      14.676  10.591  15.571  1.00 27.68
ATOM     26  CD1 ILE   193      13.327   8.117  14.795  1.00 30.01
ATOM     27  N   LYS   194      13.059  13.653  14.457  1.00 20.73
ATOM     28  CA  LYS   194      13.737  14.904  14.133  1.00 19.35
ATOM     29  C   LYS   194      12.948  15.626  13.044  1.00 17.19
ATOM     30  O   LYS   194      12.458  14.992  12.109  1.00 18.78
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ATOM     33  CD  LYS   194      17.444  15.770  13.287  1.00 21.49
ATOM     34  CE  LYS   194      18.150  17.095  12.991  1.00 21.52
ATOM     35  NZ  LYS   194      18.242  17.933  14.228  1.00 21.16
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ATOM     39  O   ALA   195      13.410  19.639  13.091  1.00 19.74
ATOM     40  CB  ALA   195      10.722  17.956  12.483  1.00 19.17
ATOM     41  N   GLY   196      12.843  19.679  10.897  1.00 15.89
ATOM     42  CA  GLY   196      13.455  21.006  10.717  1.00 15.50
ATOM     43  C   GLY   196      13.569  21.336   9.252  1.00 13.96
ATOM     44  O   GLY   196      13.456  20.449   8.386  1.00 14.30
ATOM     45  N   TYR   197      13.871  22.602   8.981  1.00 14.45
ATOM     46  CA  TYR   197      14.065  23.034   7.613  1.00 15.24
ATOM     47  C   TYR   197      15.477  22.692   7.116  1.00 15.32
ATOM     48  O   TYR   197      16.459  22.856   7.850  1.00 15.55
ATOM     49  CB  TYR   197      13.861  24.553   7.485  1.00 15.09
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ATOM     50  CG  TYR   197      12.421  24.981   7.601  1.00 15.65
ATOM     51  CD1 TYR   197      11.568  24.805   6.502  1.00 17.07
ATOM     52  CD2 TYR   197      11.909  25.556   8.768  1.00 16.88
ATOM     53  CE1 TYR   197      10.236  25.179   6.557  1.00 17.76
ATOM     54  CE2 TYR   197      10.575  25.944   8.835  1.00 19.68
ATOM     55  CZ  TYR   197       9.762  25.754   7.729  1.00 19.90
ATOM     56  OH  TYR   197       8.430  26.125   7.754  1.00 22.65
ATOM     57  N   CYS   198      15.550  22.260   5.853  1.00 14.92
ATOM     58  CA  CYS   198      16.806  22.089   5.145  1.00 14.92
ATOM     59  C   CYS   198      16.569  22.613   3.724  1.00 15.35
ATOM     60  O   CYS   198      15.409  22.792   3.334  1.00 17.54
ATOM     61  CB  CYS   198      17.257  20.640   5.009  1.00 15.37
ATOM     62  SG  CYS   198      17.671  19.831   6.580  1.00 17.71
ATOM     63  N   VAL   199      17.595  22.848   2.956  1.00 15.86
ATOM     64  CA  VAL   199      17.408  23.328   1.576  1.00 15.32
ATOM     65  C   VAL   199      17.847  22.217   0.642  1.00 16.35
ATOM     66  O   VAL   199      18.961  21.687   0.711  1.00 17.31
ATOM     67  CB  VAL   199      18.311  24.568   1.422  0.75 15.48
ATOM     68  CG1 VAL   199      18.594  24.970  -0.014  0.75 15.12
ATOM     69  CG2 VAL   199      17.645  25.746   2.148  0.75 16.22
ATOM     70  N   LYS   200      16.936  21.783  -0.218  1.00 16.07
ATOM     71  CA  LYS   200      17.176  20.584  -1.043  1.00 16.73
ATOM     72  C   LYS   200      17.145  20.934  -2.509  1.00 16.33
ATOM     73  O   LYS   200      16.517  21.908  -2.951  1.00 17.08
ATOM     74  CB  LYS   200      16.125  19.527  -0.679  1.00 19.13
ATOM     75  CG  LYS   200      14.666  19.871  -0.944  1.00 22.54
ATOM     76  CD  LYS   200      13.778  18.699  -1.307  1.00 27.53
ATOM     77  CE  LYS   200      14.105  17.912  -2.537  1.00 29.55
ATOM     78  NZ  LYS   200      13.438  18.388  -3.793  1.00 34.21
ATOM     79  N   GLN   201      17.831  20.133  -3.294  1.00 15.66
ATOM     80  CA  GLN   201      17.895  20.351  -4.730  1.00 15.70
ATOM     81  C   GLN   201      16.654  19.805  -5.416  1.00 17.09
ATOM     82  O   GLN   201      16.198  18.680  -5.130  1.00 19.12
ATOM     83  CB  GLN   201      19.166  19.698  -5.322  1.00 18.36
ATOM     84  CG  GLN   201      19.400  20.171  -6.774  1.00 20.43
ATOM     85  CD  GLN   201      20.711  19.656  -7.352  1.00 22.21
ATOM     86  OE1 GLN   201      21.654  20.378  -7.758  1.00 31.26
ATOM     87  NE2 GLN   201      20.807  18.362  -7.443  1.00 26.06
ATOM     88  N   GLY   202      16.110  20.616  -6.313  1.00 19.33
ATOM     89  CA  GLY   202      14.870  20.254  -7.016  1.00 23.01
ATOM     90  C   GLY   202      15.172  19.543  -8.312  1.00 26.26
ATOM     91  O   GLY   202      16.261  19.684  -8.879  1.00 36.17
ATOM     92  N   ALA   203      14.236  18.739  -8.772  1.00 28.02
ATOM     93  CA  ALA   203      14.519  17.979  -9.991  1.00 30.54
ATOM     94  C   ALA   203      14.293  18.809 -11.266  1.00 31.63
ATOM     95  O   ALA   203      14.993  18.534 -12.245  1.00 36.71
ATOM     96  CB  ALA   203      13.661  16.722 -10.030  1.00 33.09
ATOM     97  N   VAL   204      13.365  19.762 -11.273  1.00 30.97
ATOM     98  CA  VAL   204      12.943  20.349 -12.551  1.00 32.03
ATOM     99  C   VAL   204      14.088  21.087 -13.239  1.00 29.36
ATOM    100  O   VAL   204      14.389  20.921 -14.416  1.00 35.33
ATOM    101  CB  VAL   204      11.765  21.340 -12.375  1.00 34.35
ATOM    102  CG1 VAL   204      11.328  21.918 -13.724  1.00 40.14
ATOM    103  CG2 VAL   204      10.613  20.663 -11.639  1.00 39.80
ATOM    104  N   MET   205      14.772  21.937 -12.506  1.00 25.09
ATOM    105  CA  MET   205      15.844  22.769 -13.035  1.00 21.04
ATOM    106  C   MET   205      17.053  22.751 -12.102  1.00 19.16
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ATOM    107  O   MET   205      18.008  23.542 -12.286  1.00 19.75
ATOM    108  CB  MET   205      15.358  24.212 -13.239  1.00 20.91
ATOM    109  CG  MET   205      14.356  24.297 -14.410  1.00 21.61
ATOM    110  SD  MET   205      13.932  26.026 -14.752  1.00 21.67
ATOM    111  CE  MET   205      12.578  25.801 -15.895  1.00 22.26
ATOM    112  N   LYS   206      17.064  21.920 -11.067  1.00 19.57
ATOM    113  CA  LYS   206      18.138  21.774 -10.103  1.00 20.94
ATOM    114  C   LYS   206      18.399  23.025  -9.276  1.00 19.57
ATOM    115  O   LYS   206      19.533  23.315  -8.852  1.00 21.42
ATOM    116  CB  LYS   206      19.420  21.322 -10.830  1.00 24.94
ATOM    117  CG  LYS   206      19.146  20.075 -11.683  1.00 32.02
ATOM    118  CD  LYS   206      20.136  18.959 -11.438  1.00 38.26
ATOM    119  CE  LYS   206      19.618  17.606 -11.911  1.00 40.49
ATOM    120  NZ  LYS   206      18.367  17.728 -12.734  1.00 52.62
ATOM    121  N   ASN   207      17.312  23.771  -9.011  1.00 17.51
ATOM    122  CA  ASN   207      17.393  24.862  -8.026  1.00 16.08
ATOM    123  C   ASN   207      17.248  24.301  -6.622  1.00 17.69
ATOM    124  O   ASN   207      16.945  23.129  -6.411  1.00 22.08
ATOM    125  CB  ASN   207      16.334  25.926  -8.325  1.00 17.06
ATOM    126  CG  ASN   207      16.699  26.697  -9.591  1.00 15.66
ATOM    127  OD1 ASN   207      17.874  27.002  -9.776  1.00 17.22
ATOM    128  ND2 ASN   207      15.700  26.983 -10.412  1.00 17.30
ATOM    129  N   TRP   208      17.500  25.171  -5.645  1.00 18.51
ATOM    130  CA  TRP   208      17.549  24.756  -4.253  1.00 18.10
ATOM    131  C   TRP   208      16.403  25.431  -3.517  1.00 18.79
ATOM    132  O   TRP   208      16.189  26.639  -3.710  1.00 24.72
ATOM    133  CB  TRP   208      18.908  25.137  -3.637  1.00 19.18
ATOM    134  CG  TRP   208      20.046  24.404  -4.279  1.00 19.28
ATOM    135  CD1 TRP   208      20.727  24.701  -5.418  1.00 21.08
ATOM    136  CD2 TRP   208      20.627  23.202  -3.782  1.00 18.40
ATOM    137  NE1 TRP   208      21.708  23.782  -5.682  1.00 21.34
ATOM    138  CE2 TRP   208      21.670  22.834  -4.680  1.00 19.36
ATOM    139  CE3 TRP   208      20.371  22.403  -2.649  1.00 18.06
ATOM    140  CZ2 TRP   208      22.444  21.692  -4.474  1.00 19.16
ATOM    141  CZ3 TRP   208      21.165  21.277  -2.478  1.00 19.17
ATOM    142  CH2 TRP   208      22.190  20.912  -3.364  1.00 19.13
ATOM    143  N   LYS   209      15.684  24.654  -2.730  1.00 19.05
ATOM    144  CA  LYS   209      14.526  25.200  -2.045  1.00 21.22
ATOM    145  C   LYS   209      14.430  24.767  -0.600  1.00 18.50
ATOM    146  O   LYS   209      14.674  23.616  -0.277  1.00 16.89
ATOM    147  CB  LYS   209      13.239  24.774  -2.764  1.00 26.16
ATOM    148  CG  LYS   209      13.188  25.288  -4.190  1.00 31.25
ATOM    149  CD  LYS   209      12.173  24.581  -5.033  1.00 33.26
ATOM    150  CE  LYS   209      12.388  24.873  -6.518  0.75 32.48
ATOM    151  NZ  LYS   209      12.486  26.327  -6.778  0.75 26.04
ATOM    152  N   ARG   210      14.038  25.729   0.235  1.00 17.98
ATOM    153  CA  ARG   210      13.842  25.381   1.652  1.00 17.79
ATOM    154  C   ARG   210      12.579  24.575   1.844  1.00 16.62
ATOM    155  O   ARG   210      11.504  25.021   1.412  1.00 20.55
ATOM    156  CB  ARG   210      13.827  26.688   2.452  1.00 20.45
ATOM    157  CG  ARG   210      13.823  26.353   3.956  1.00 24.63
ATOM    158  CD  ARG   210      14.207  27.604   4.733  1.00 31.80
ATOM    159  NE  ARG   210      13.419  27.632   5.935  1.00 38.28
ATOM    160  CZ  ARG   210      12.401  28.468   6.103  1.00 35.20
ATOM    161  NH1 ARG   210      12.118  29.298   5.107  1.00 46.66
ATOM    162  NH2 ARG   210      11.749  28.412   7.225  1.00 30.88
ATOM    163  N   ARG   211      12.730  23.385   2.451  1.00 15.34
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ATOM	164	CA	ARG	211	11.607	22.499	2.709	1.00	15.17
ATOM	165	C	ARG	211	11.698	22.015	4.152	1.00	14.69
ATOM	166	O	ARG	211	12.769	22.016	4.755	1.00	15.04
ATOM	167	CB	ARG	211	11.568	21.273	1.776	1.00	16.36
ATOM	168	CG	ARG	211	11.392	21.641	0.285	1.00	19.47
ATOM	169	CD	ARG	211	10.051	22.310	0.067	1.00	22.23
ATOM	170	NE	ARG	211	9.776	22.671	-1.322	1.00	26.63
ATOM	171	CZ	ARG	211	9.377	23.867	-1.730	1.00	30.35
ATOM	172	NH1	ARG	211	9.230	24.813	-0.808	1.00	34.82
ATOM	173	NH2	ARG	211	9.142	24.099	-3.002	1.00	33.50
ATOM	174	N	TYR	212	10.550	21.608	4.707	1.00	15.01
ATOM	175	CA	TYR	212	10.503	21.141	6.088	1.00	14.62
ATOM	176	C	TYR	212	10.615	19.626	6.119	1.00	14.04
ATOM	177	O	TYR	212	9.752	18.950	5.564	1.00	16.28
ATOM	178	CB	TYR	212	9.189	21.613	6.711	1.00	16.07
ATOM	179	CG	TYR	212	9.084	21.342	8.193	1.00	17.06
ATOM	180	CD1	TYR	212	9.792	22.121	9.105	1.00	18.69
ATOM	181	CD2	TYR	212	8.276	20.295	8.662	1.00	18.27
ATOM	182	CE1	TYR	212	9.705	21.870	10.476	1.00	21.10
ATOM	183	CE2	TYR	212	8.185	20.059	10.027	1.00	21.25
ATOM	184	CZ	TYR	212	8.891	20.840	10.917	1.00	22.14
ATOM	185	OH	TYR	212	8.770	20.581	12.264	1.00	27.64
ATOM	186	N	PHE	213	11.670	19.135	6.715	1.00	14.27
ATOM	187	CA	PHE	213	11.927	17.693	6.770	1.00	14.15
ATOM	188	C	PHE	213	11.453	17.106	8.102	1.00	14.31
ATOM	189	O	PHE	213	11.544	17.760	9.145	1.00	16.58
ATOM	190	CB	PHE	213	13.416	17.394	6.606	1.00	14.35
ATOM	191	CG	PHE	213	13.866	17.460	5.144	1.00	14.82
ATOM	192	CD1	PHE	213	14.018	18.669	4.483	1.00	15.15
ATOM	193	CD2	PHE	213	14.125	16.279	4.449	1.00	15.27
ATOM	194	CE1	PHE	213	14.435	18.716	3.158	1.00	15.87
ATOM	195	CE2	PHE	213	14.517	16.342	3.112	1.00	15.74
ATOM	196	CZ	PHE	213	14.661	17.549	2.463	1.00	16.14
ATOM	197	N	GLN	214	10.972	15.863	8.043	1.00	15.31
ATOM	198	CA	GLN	214	10.450	15.152	9.186	1.00	16.72
ATOM	199	C	GLN	214	10.858	13.683	9.143	1.00	16.94
ATOM	200	O	GLN	214	10.530	13.027	8.146	1.00	19.61
ATOM	201	CB	GLN	214	8.926	15.328	9.212	0.50	19.97
ATOM	202	CG	GLN	214	8.317	14.841	10.518	0.50	20.22
ATOM	203	CD	GLN	214	6.861	15.250	10.637	0.50	22.42
ATOM	204	OE1	GLN	214	6.558	16.400	10.940	0.50	26.77
ATOM	205	NE2	GLN	214	5.934	14.323	10.392	0.50	26.32
ATOM	206	N	LEU	215	11.536	13.221	10.167	1.00	17.75
ATOM	207	CA	LEU	215	11.959	11.828	10.274	1.00	18.02
ATOM	208	C	LEU	215	11.168	11.156	11.364	1.00	19.64
ATOM	209	O	LEU	215	11.073	11.704	12.467	1.00	21.34
ATOM	210	CB	LEU	215	13.451	11.790	10.638	1.00	18.12
ATOM	211	CG	LEU	215	14.041	10.453	11.071	1.00	19.15
ATOM	212	CD1	LEU	215	14.253	9.505	9.868	1.00	21.88
ATOM	213	CD2	LEU	215	15.360	10.631	11.815	1.00	20.64
ATOM	214	N	ASP	216	10.608	9.996	11.063	1.00	21.87
ATOM	215	CA	ASP	216	10.039	9.159	12.119	1.00	25.38
ATOM	216	C	ASP	216	10.658	7.761	12.019	1.00	26.69
ATOM	217	O	ASP	216	11.633	7.588	11.308	1.00	26.09
ATOM	218	CB	ASP	216	8.514	9.158	12.061	1.00	27.83
ATOM	219	CG	ASP	216	7.905	8.474	10.865	1.00	29.15
ATOM	220	OD1	ASP	216	8.552	7.641	10.210	1.00	27.95

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ATOM	221	OD2	ASP	216	6.736	8.756	10.570	1.00	33.08
ATOM	222	N	GLU	217	10.077	6.803	12.744	1.00	29.59
ATOM	223	CA	GLU	217	10.807	5.517	12.788	1.00	31.83
ATOM	224	C	GLU	217	10.824	4.788	11.454	1.00	30.96
ATOM	225	O	GLU	217	11.635	3.858	11.308	1.00	32.47
ATOM	226	CB	GLU	217	10.192	4.646	13.893	1.00	35.70
ATOM	227	CG	GLU	217	8.858	4.021	13.514	1.00	42.54
ATOM	228	CD	GLU	217	8.330	3.126	14.621	0.50	45.53
ATOM	229	OE1	GLU	217	8.998	3.088	15.687	0.50	45.76
ATOM	230	OE2	GLU	217	7.277	2.472	14.435	0.50	48.36
ATOM	231	N	ASN	218	9.960	5.156	10.502	1.00	29.91
ATOM	232	CA	ASN	218	9.867	4.389	9.255	1.00	30.36
ATOM	233	C	ASN	218	10.130	5.172	7.993	1.00	25.95
ATOM	234	O	ASN	218	10.330	4.597	6.933	1.00	25.15
ATOM	235	CB	ASN	218	8.444	3.816	9.102	1.00	34.55
ATOM	236	CG	ASN	218	8.165	2.730	10.120	1.00	38.61
ATOM	237	OD1	ASN	218	7.101	2.698	10.724	1.00	46.88
ATOM	238	ND2	ASN	218	9.162	1.865	10.279	1.00	41.31
ATOM	239	N	THR	219	10.087	6.513	8.074	1.00	23.80
ATOM	240	CA	THR	219	10.149	7.349	6.904	1.00	22.33
ATOM	241	C	THR	219	10.894	8.667	7.184	1.00	19.30
ATOM	242	O	THR	219	10.939	9.145	8.310	1.00	20.69
ATOM	243	CB	THR	219	8.758	7.738	6.351	1.00	25.06
ATOM	244	OG1	THR	219	8.124	8.622	7.284	1.00	30.26
ATOM	245	CG2	THR	219	7.792	6.566	6.162	1.00	27.15
ATOM	246	N	ILE	220	11.439	9.236	6.092	1.00	18.70
ATOM	247	CA	ILE	220	11.786	10.641	6.112	1.00	18.69
ATOM	248	C	ILE	220	11.033	11.286	4.963	1.00	19.99
ATOM	249	O	ILE	220	11.042	10.828	3.820	1.00	21.22
ATOM	250	CB	ILE	220	13.323	10.875	6.075	1.00	20.88
ATOM	251	CG1	ILE	220	13.641	12.356	6.275	1.00	22.53
ATOM	252	CG2	ILE	220	13.963	10.267	4.831	1.00	22.44
ATOM	253	CD1	ILE	220	15.061	12.730	6.534	1.00	24.03
ATOM	254	N	GLY	221	10.326	12.366	5.294	1.00	20.37
ATOM	255	CA	GLY	221	9.554	13.075	4.265	1.00	21.16
ATOM	256	C	GLY	221	9.836	14.559	4.353	1.00	18.54
ATOM	257	O	GLY	221	10.420	15.082	5.327	1.00	19.01
ATOM	258	N	TYR	222	9.420	15.256	3.300	1.00	16.85
ATOM	259	CA	TYR	222	9.521	16.715	3.374	1.00	16.44
ATOM	260	C	TYR	222	8.221	17.337	2.875	1.00	16.89
ATOM	261	O	TYR	222	7.472	16.733	2.096	1.00	18.99
ATOM	262	CB	TYR	222	10.758	17.250	2.640	1.00	17.17
ATOM	263	CG	TYR	222	10.926	16.825	1.202	1.00	17.84
ATOM	264	CD1	TYR	222	10.326	17.547	0.148	1.00	19.09
ATOM	265	CD2	TYR	222	11.667	15.711	0.851	1.00	19.62
ATOM	266	CE1	TYR	222	10.469	17.164	-1.180	1.00	20.54
ATOM	267	CE2	TYR	222	11.819	15.333	-0.470	1.00	20.82
ATOM	268	CZ	TYR	222	11.224	16.044	-1.481	1.00	21.19
ATOM	269	OH	TYR	222	11.410	15.635	-2.774	1.00	24.70
ATOM	270	N	PHE	223	8.032	18.539	3.410	1.00	16.71
ATOM	271	CA	PHE	223	6.815	19.317	3.249	1.00	18.24
ATOM	272	C	PHE	223	7.150	20.714	2.737	1.00	18.27
ATOM	273	O	PHE	223	8.282	21.196	2.909	1.00	18.50
ATOM	274	CB	PHE	223	6.106	19.488	4.587	1.00	19.17
ATOM	275	CG	PHE	223	5.701	18.213	5.303	1.00	20.05
ATOM	276	CD1	PHE	223	6.634	17.382	5.892	1.00	20.99
ATOM	277	CD2	PHE	223	4.360	17.870	5.388	1.00	21.43

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ATOM    278  CE1 PHE   223       6.233  16.245   6.552  1.00 23.44
ATOM    279  CE2 PHE   223       3.967  16.733   6.054  1.00 23.56
ATOM    280  CZ  PHE   223       4.891  15.902   6.651  1.00 24.88
ATOM    281  N   LYS   224       6.182  21.362   2.119  1.00 20.33
ATOM    282  CA  LYS   224       6.418  22.744   1.661  1.00 22.12
ATOM    283  C   LYS   224       6.867  23.664   2.796  1.00 23.32
ATOM    284  O   LYS   224       7.748  24.515   2.608  1.00 25.85
ATOM    285  CB  LYS   224       5.143  23.310   1.017  1.00 25.60
ATOM    286  CG  LYS   224       5.482  24.519   0.164  1.00 30.45
ATOM    287  CD  LYS   224       4.383  24.816  -0.844  0.00 30.55
ATOM    288  CE  LYS   224       3.364  25.792  -0.281  0.00 30.54
ATOM    289  NZ  LYS   224       2.572  25.190   0.828  0.00 30.54
ATOM    290  N   SER   225       6.266  23.494   3.976  1.00 22.08
ATOM    291  CA  SER   225       6.580  24.242   5.195  1.00 23.74
ATOM    292  C   SER   225       6.133  23.436   6.398  1.00 24.03
ATOM    293  O   SER   225       5.515  22.364   6.281  1.00 23.46
ATOM    294  CB  SER   225       5.921  25.637   5.173  1.00 27.63
ATOM    295  OG  SER   225       4.566  25.542   5.542  1.00 32.34
ATOM    296  N   GLU   226       6.425  23.958   7.584  1.00 24.05
ATOM    297  CA  GLU   226       6.026  23.313   8.818  1.00 24.93
ATOM    298  C   GLU   226       4.521  23.276   9.011  1.00 26.76
ATOM    299  O   GLU   226       3.992  22.510   9.827  1.00 31.39
ATOM    300  CB  GLU   226       6.693  24.067   9.966  1.00 25.73
ATOM    301  CG  GLU   226       6.437  23.433  11.310  1.00 28.90
ATOM    302  CD  GLU   226       7.125  24.145  12.446  1.00 32.94
ATOM    303  OE1 GLU   226       7.586  25.282  12.211  1.00 40.00
ATOM    304  OE2 GLU   226       7.218  23.557  13.543  1.00 40.96
ATOM    305  N   LEU   227       3.774  24.103   8.271  1.00 28.94
ATOM    306  CA  LEU   227       2.335  24.162   8.478  1.00 31.01
ATOM    307  C   LEU   227       1.570  23.102   7.707  1.00 30.69
ATOM    308  O   LEU   227       0.465  22.756   8.135  1.00 33.63
ATOM    309  CB  LEU   227       1.799  25.533   8.044  1.00 33.52
ATOM    310  CG  LEU   227       2.502  26.780   8.554  1.00 37.29
ATOM    311  CD1 LEU   227       2.594  27.853   7.461  1.00 43.93
ATOM    312  CD2 LEU   227       1.790  27.379   9.736  1.00 44.26
ATOM    313  N   GLU   228       2.165  22.640   6.609  1.00 29.16
ATOM    314  CA  GLU   228       1.422  21.759   5.708  1.00 30.92
ATOM    315  C   GLU   228       1.085  20.416   6.336  1.00 32.97
ATOM    316  O   GLU   228       1.863  19.800   7.068  1.00 33.36
ATOM    317  CB  GLU   228       2.199  21.483   4.416  1.00 33.73
ATOM    318  CG  GLU   228       1.842  22.478   3.316  0.50 36.19
ATOM    319  CD  GLU   228       2.482  23.827   3.584  0.50 35.91
ATOM    320  OE1 GLU   228       3.577  23.833   4.176  0.50 35.94
ATOM    321  OE2 GLU   228       1.910  24.878   3.220  0.50 38.74
ATOM    322  N   LYS   229      -0.134  19.961   6.017  1.00 33.57
ATOM    323  CA  LYS   229      -0.531  18.685   6.603  1.00 36.41
ATOM    324  C   LYS   229      -0.018  17.526   5.770  1.00 33.58
ATOM    325  O   LYS   229       0.271  16.460   6.309  1.00 37.12
ATOM    326  CB  LYS   229      -2.053  18.626   6.742  1.00 39.66
ATOM    327  CG  LYS   229      -2.571  19.499   7.886  1.00 42.62
ATOM    328  CD  LYS   229      -3.633  18.741   8.676  1.00 45.48
ATOM    329  CE  LYS   229      -4.142  19.499   9.890  1.00 47.11
ATOM    330  NZ  LYS   229      -4.517  18.569  11.000  1.00 50.11
ATOM    331  N   GLU   230       0.093  17.714   4.466  1.00 30.84
ATOM    332  CA  GLU   230       0.481  16.612   3.592  1.00 31.89
ATOM    333  C   GLU   230       1.893  16.810   3.066  1.00 28.41
ATOM    334  O   GLU   230       2.191  17.917   2.654  1.00 27.47
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ATOM    335  CB  GLU   230      -0.522  16.488   2.431  1.00 35.75
ATOM    336  CG  GLU   230      -1.914  16.083   2.910  1.00 39.89
ATOM    337  CD  GLU   230      -1.782  14.912   3.874  1.00 46.50
ATOM    338  OE1 GLU   230      -1.081  13.940   3.505  1.00 55.46
ATOM    339  OE2 GLU   230      -2.360  14.960   4.982  1.00 56.83
ATOM    340  N   PRO   231       2.724  15.776   3.079  1.00 26.68
ATOM    341  CA  PRO   231       4.080  15.952   2.542  1.00 23.82
ATOM    342  C   PRO   231       4.126  16.031   1.017  1.00 24.10
ATOM    343  O   PRO   231       3.219  15.600   0.300  1.00 29.95
ATOM    344  CB  PRO   231       4.814  14.693   2.998  1.00 25.42
ATOM    345  CG  PRO   231       3.714  13.671   3.122  1.00 27.85
ATOM    346  CD  PRO   231       2.476  14.418   3.593  1.00 27.93
ATOM    347  N   LEU   232       5.217  16.595   0.509  1.00 22.66
ATOM    348  CA  LEU   232       5.506  16.603  -0.926  1.00 22.49
ATOM    349  C   LEU   232       6.045  15.248  -1.335  1.00 22.14
ATOM    350  O   LEU   232       5.766  14.747  -2.431  1.00 24.82
ATOM    351  CB  LEU   232       6.546  17.653  -1.244  1.00 22.61
ATOM    352  CG  LEU   232       6.051  19.091  -1.071  1.00 23.81
ATOM    353  CD1 LEU   232       7.206  20.071  -1.116  1.00 24.77
ATOM    354  CD2 LEU   232       5.026  19.470  -2.143  1.00 32.23
ATOM    355  N   ARG   233       6.846  14.642  -0.465  1.00 21.35
ATOM    356  CA  ARG   233       7.417  13.321  -0.768  1.00 21.94
ATOM    357  C   ARG   233       7.699  12.574   0.527  1.00 19.89
ATOM    358  O   ARG   233       8.128  13.220   1.488  1.00 20.49
ATOM    359  CB  ARG   233       8.680  13.517  -1.609  1.00 25.16
ATOM    360  CG  ARG   233       9.415  12.233  -1.924  1.00 30.23
ATOM    361  CD  ARG   233      10.652  12.482  -2.762  1.00 34.82
ATOM    362  NE  ARG   233      10.246  12.681  -4.153  1.00 38.87
ATOM    363  CZ  ARG   233       9.994  11.535  -4.823  1.00 42.69
ATOM    364  NH1 ARG   233      10.123  10.382  -4.164  1.00 49.40
ATOM    365  NH2 ARG   233       9.618  11.554  -6.085  1.00 49.87
ATOM    366  N   VAL   234       7.472  11.267   0.522  1.00 21.05
ATOM    367  CA  VAL   234       7.872  10.405   1.627  1.00 21.92
ATOM    368  C   VAL   234       8.869   9.399   1.122  1.00 21.67
ATOM    369  O   VAL   234       8.628   8.783   0.061  1.00 26.32
ATOM    370  CB  VAL   234       6.622   9.729   2.210  1.00 26.64
ATOM    371  CG1 VAL   234       6.990   8.702   3.266  1.00 31.57
ATOM    372  CG2 VAL   234       5.692  10.811   2.766  1.00 28.45
ATOM    373  N   ILE   235       9.955   9.258   1.843  1.00 20.34
ATOM    374  CA  ILE   235      10.983   8.285   1.499  1.00 21.05
ATOM    375  C   ILE   235      10.995   7.227   2.583  1.00 20.54
ATOM    376  O   ILE   235      11.367   7.460   3.730  1.00 22.45
ATOM    377  CB  ILE   235      12.362   8.963   1.389  1.00 20.88
ATOM    378  CG1 ILE   235      12.407  10.090   0.337  1.00 22.52
ATOM    379  CG2 ILE   235      13.486   7.961   1.121  1.00 22.62
ATOM    380  CD1 ILE   235      13.561  11.079   0.480  1.00 25.24
ATOM    381  N   PRO   236      10.604   6.016   2.289  1.00 23.14
ATOM    382  CA  PRO   236      10.696   4.955   3.291  1.00 23.97
ATOM    383  C   PRO   236      12.165   4.683   3.629  1.00 23.18
ATOM    384  O   PRO   236      13.033   4.579   2.769  1.00 22.92
ATOM    385  CB  PRO   236      10.061   3.745   2.608  1.00 26.55
ATOM    386  CG  PRO   236       9.229   4.355   1.504  1.00 28.05
ATOM    387  CD  PRO   236      10.039   5.551   1.008  1.00 25.69
ATOM    388  N   LEU   237      12.500   4.571   4.905  1.00 23.30
ATOM    389  CA  LEU   237      13.882   4.353   5.308  1.00 22.75
ATOM    390  C   LEU   237      14.395   3.040   4.758  1.00 22.07
ATOM    391  O   LEU   237      15.593   2.916   4.526  1.00 22.91
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ATOM	392	CB	LEU	237	14.019	4.350	6.840	1.00	23.32
ATOM	393	CG	LEU	237	13.742	5.725	7.498	1.00	23.19
ATOM	394	CD1	LEU	237	13.757	5.560	9.014	1.00	26.51
ATOM	395	CD2	LEU	237	14.755	6.753	7.028	1.00	23.10
ATOM	396	N	LYS	238	13.513	2.036	4.550	1.00	23.47
ATOM	397	CA	LYS	238	13.946	0.760	4.004	1.00	24.93
ATOM	398	C	LYS	238	14.478	0.890	2.580	1.00	23.62
ATOM	399	O	LYS	238	15.178	0.000	2.110	1.00	25.29
ATOM	400	CB	LYS	238	12.798	-0.263	4.013	1.00	27.48
ATOM	401	CG	LYS	238	12.648	-0.763	5.446	1.00	31.27
ATOM	402	CD	LYS	238	11.550	-1.805	5.512	1.00	34.54
ATOM	403	CE	LYS	238	10.222	-1.172	5.116	0.50	35.66
ATOM	404	NZ	LYS	238	9.965	-1.169	3.657	0.50	34.76
ATOM	405	N	GLU	239	14.167	2.007	1.920	1.00	24.02
ATOM	406	CA	GLU	239	14.629	2.259	0.555	1.00	23.02
ATOM	407	C	GLU	239	15.877	3.125	0.565	1.00	22.35
ATOM	408	O	GLU	239	16.390	3.468	-0.508	1.00	23.15
ATOM	409	CB	GLU	239	13.510	2.911	-0.263	1.00	25.73
ATOM	410	CG	GLU	239	12.249	2.055	-0.285	1.00	27.65
ATOM	411	CD	GLU	239	11.160	2.601	-1.188	1.00	30.52
ATOM	412	OE1	GLU	239	11.316	3.669	-1.808	1.00	33.05
ATOM	413	OE2	GLU	239	10.096	1.965	-1.294	1.00	39.53
ATOM	414	N	VAL	240	16.370	3.477	1.753	1.00	22.16
ATOM	415	CA	VAL	240	17.592	4.300	1.847	1.00	20.78
ATOM	416	C	VAL	240	18.835	3.391	1.892	1.00	22.35
ATOM	417	O	VAL	240	19.015	2.617	2.830	1.00	24.58
ATOM	418	CB	VAL	240	17.563	5.252	3.050	1.00	19.93
ATOM	419	CG1	VAL	240	18.870	6.007	3.177	1.00	20.96
ATOM	420	CG2	VAL	240	16.376	6.218	2.900	1.00	20.85
ATOM	421	N	HIS	241	19.662	3.497	0.858	1.00	22.88
ATOM	422	CA	HIS	241	20.864	2.686	0.749	1.00	24.02
ATOM	423	C	HIS	241	22.004	3.345	1.505	1.00	25.03
ATOM	424	O	HIS	241	22.837	2.658	2.096	1.00	28.89
ATOM	425	CB	HIS	241	21.262	2.446	-0.710	1.00	24.65
ATOM	426	CG	HIS	241	20.202	1.895	-1.602	1.00	26.10
ATOM	427	ND1	HIS	241	19.466	0.754	-1.323	1.00	28.22
ATOM	428	CD2	HIS	241	19.749	2.317	-2.803	1.00	26.54
ATOM	429	CE1	HIS	241	18.603	0.526	-2.320	1.00	29.54
ATOM	430	NE2	HIS	241	18.768	1.473	-3.221	1.00	27.97
ATOM	431	N	LYS	242	22.088	4.679	1.525	1.00	24.57
ATOM	432	CA	LYS	242	23.098	5.432	2.263	1.00	24.90
ATOM	433	C	LYS	242	22.553	6.771	2.713	1.00	21.68
ATOM	434	O	LYS	242	21.742	7.384	1.979	1.00	21.96
ATOM	435	CB	LYS	242	24.325	5.718	1.393	1.00	29.67
ATOM	436	CG	LYS	242	24.961	4.508	0.703	1.00	36.67
ATOM	437	CD	LYS	242	25.229	4.788	-0.763	1.00	40.74
ATOM	438	CE	LYS	242	26.280	5.863	-0.941	1.00	43.61
ATOM	439	NZ	LYS	242	27.369	5.554	-1.899	1.00	48.58
ATOM	440	N	VAL	243	22.993	7.245	3.868	1.00	20.61
ATOM	441	CA	VAL	243	22.815	8.635	4.279	1.00	20.85
ATOM	442	C	VAL	243	24.171	9.107	4.775	1.00	21.31
ATOM	443	O	VAL	243	24.813	8.510	5.652	1.00	24.65
ATOM	444	CB	VAL	243	21.653	8.794	5.287	0.75	20.04
ATOM	445	CG1	VAL	243	21.797	7.913	6.510	0.75	21.76
ATOM	446	CG2	VAL	243	21.495	10.277	5.651	0.75	18.19
ATOM	447	N	GLN	244	24.671	10.190	4.177	1.00	21.27
ATOM	448	CA	GLN	244	26.069	10.523	4.429	1.00	22.78

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ATOM	449	C	GLN	244	26.365	11.933	3.940	1.00	22.13
ATOM	450	O	GLN	244	25.626	12.506	3.139	1.00	21.20
ATOM	451	CB	GLN	244	26.988	9.520	3.709	1.00	25.50
ATOM	452	CG	GLN	244	26.812	9.584	2.187	1.00	28.52
ATOM	453	CD	GLN	244	27.536	8.461	1.469	1.00	31.26
ATOM	454	OE1	GLN	244	28.123	7.576	2.082	1.00	35.37
ATOM	455	NE2	GLN	244	27.504	8.477	0.132	1.00	32.99
ATOM	456	N	GLU	245	27.475	12.498	4.390	1.00	23.01
ATOM	457	CA	GLU	245	27.972	13.741	3.839	1.00	23.76
ATOM	458	C	GLU	245	28.158	13.625	2.326	1.00	25.04
ATOM	459	O	GLU	245	28.645	12.602	1.839	1.00	27.71
ATOM	460	CB	GLU	245	29.294	14.167	4.466	0.50	27.35
ATOM	461	CG	GLU	245	29.282	14.240	5.982	0.50	27.40
ATOM	462	CD	GLU	245	30.593	14.842	6.477	0.50	30.22
ATOM	463	OE1	GLU	245	31.189	15.626	5.717	0.50	29.59
ATOM	464	OE2	GLU	245	30.966	14.490	7.616	0.50	37.80
ATOM	465	N	CYS	246	27.802	14.684	1.591	1.00	25.16
ATOM	466	CA	CYS	246	27.887	14.569	0.136	1.00	26.20
ATOM	467	C	CYS	246	29.288	14.937	-0.317	1.00	27.99
ATOM	468	O	CYS	246	29.767	16.063	-0.127	1.00	32.42
ATOM	469	CB	CYS	246	26.764	15.430	-0.484	1.00	25.05
ATOM	470	SG	CYS	246	26.892	15.540	-2.279	1.00	29.58
ATOM	471	N	LYS	247	30.009	13.988	-0.934	1.00	30.69
ATOM	472	CA	LYS	247	31.387	14.289	-1.330	1.00	34.72
ATOM	473	C	LYS	247	31.466	15.247	-2.518	1.00	36.20
ATOM	474	O	LYS	247	32.541	15.684	-2.911	1.00	40.58
ATOM	475	CB	LYS	247	32.109	12.972	-1.631	1.00	37.54
ATOM	476	CG	LYS	247	32.155	12.084	-0.389	1.00	40.02
ATOM	477	CD	LYS	247	32.977	12.737	0.712	0.00	40.02
ATOM	478	CE	LYS	247	32.176	12.862	1.998	0.00	40.02
ATOM	479	NZ	LYS	247	33.049	13.132	3.173	0.00	40.02
ATOM	480	N	GLN	248	30.334	15.607	-3.110	1.00	36.37
ATOM	481	CA	GLN	248	30.261	16.518	-4.245	1.00	37.18
ATOM	482	C	GLN	248	29.866	17.919	-3.773	1.00	37.21
ATOM	483	O	GLN	248	29.554	18.730	-4.652	1.00	38.45
ATOM	484	CB	GLN	248	29.263	16.065	-5.318	1.00	42.15
ATOM	485	CG	GLN	248	29.645	14.811	-6.105	1.00	45.41
ATOM	486	CD	GLN	248	29.871	13.581	-5.248	1.00	47.22
ATOM	487	OE1	GLN	248	28.957	12.938	-4.734	1.00	55.08
ATOM	488	NE2	GLN	248	31.154	13.235	-5.054	1.00	53.74
ATOM	489	N	SER	249	29.894	18.138	-2.460	1.00	36.25
ATOM	490	CA	SER	249	29.543	19.402	-1.825	1.00	36.53
ATOM	491	C	SER	249	30.364	20.534	-2.417	1.00	36.69
ATOM	492	O	SER	249	29.863	21.635	-2.693	1.00	34.51
ATOM	493	CB	SER	249	29.747	19.318	-0.308	0.50	36.02
ATOM	494	OG	SER	249	29.791	20.614	0.277	0.50	37.35
ATOM	495	N	ASP	250	31.652	20.294	-2.656	1.00	38.64
ATOM	496	CA	ASP	250	32.421	21.404	-3.239	1.00	41.19
ATOM	497	C	ASP	250	31.972	21.792	-4.632	1.00	39.28
ATOM	498	O	ASP	250	31.726	22.972	-4.932	1.00	42.28
ATOM	499	CB	ASP	250	33.906	20.989	-3.215	1.00	44.62
ATOM	500	CG	ASP	250	34.280	20.816	-1.741	1.00	48.27
ATOM	501	OD1	ASP	250	33.539	21.424	-0.941	1.00	56.18
ATOM	502	OD2	ASP	250	35.255	20.121	-1.421	1.00	54.93
ATOM	503	N	ILE	251	31.840	20.827	-5.537	1.00	39.32
ATOM	504	CA	ILE	251	31.356	21.122	-6.877	1.00	38.60
ATOM	505	C	ILE	251	29.940	21.677	-6.909	1.00	36.05

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ATOM	506	O	ILE	251	29.640	22.469	-7.814	1.00	40.66
ATOM	507	CB	ILE	251	31.403	19.824	-7.712	1.00	43.41
ATOM	508	CG1	ILE	251	32.794	19.180	-7.687	1.00	46.39
ATOM	509	CG2	ILE	251	30.893	20.056	-9.120	1.00	45.12
ATOM	510	CD1	ILE	251	32.771	17.836	-6.975	1.00	52.78
ATOM	511	N	MET	252	29.052	21.292	-5.985	1.00	32.96
ATOM	512	CA	MET	252	27.673	21.793	-6.019	1.00	32.37
ATOM	513	C	MET	252	27.529	23.128	-5.315	1.00	32.66
ATOM	514	O	MET	252	26.472	23.767	-5.212	1.00	32.59
ATOM	515	CB	MET	252	26.729	20.759	-5.380	1.00	32.57
ATOM	516	CG	MET	252	26.827	19.402	-6.070	1.00	33.97
ATOM	517	SD	MET	252	25.747	18.217	-5.233	1.00	35.26
ATOM	518	CE	MET	252	24.156	18.632	-5.926	1.00	36.15
ATOM	519	N	MET	253	28.687	23.534	-4.802	1.00	32.39
ATOM	520	CA	MET	253	28.828	24.816	-4.161	1.00	34.19
ATOM	521	C	MET	253	27.896	24.993	-2.985	1.00	31.82
ATOM	522	O	MET	253	27.307	26.062	-2.851	1.00	36.91
ATOM	523	CB	MET	253	28.570	25.920	-5.212	1.00	37.81
ATOM	524	CG	MET	253	29.800	26.741	-5.550	0.50	40.02
ATOM	525	SD	MET	253	31.120	25.703	-6.199	0.50	48.08
ATOM	526	CE	MET	253	32.278	25.735	-4.831	0.50	60.37
ATOM	527	N	ARG	254	27.776	23.948	-2.160	1.00	28.76
ATOM	528	CA	ARG	254	26.837	24.014	-1.034	1.00	25.65
ATOM	529	C	ARG	254	27.521	23.447	0.209	1.00	25.20
ATOM	530	O	ARG	254	27.817	22.256	0.222	1.00	28.32
ATOM	531	CB	ARG	254	25.554	23.228	-1.275	1.00	26.01
ATOM	532	CG	ARG	254	24.669	23.782	-2.370	1.00	26.37
ATOM	533	CD	ARG	254	23.701	24.779	-1.795	1.00	28.55
ATOM	534	NE	ARG	254	23.205	25.705	-2.817	1.00	28.38
ATOM	535	CZ	ARG	254	22.245	26.584	-2.523	1.00	28.01
ATOM	536	NH1	ARG	254	21.732	26.617	-1.296	1.00	27.15
ATOM	537	NH2	ARG	254	21.816	27.417	-3.475	1.00	30.49
ATOM	538	N	ASP	255	27.734	24.285	1.206	1.00	25.35
ATOM	539	CA	ASP	255	28.257	23.819	2.479	1.00	26.38
ATOM	540	C	ASP	255	27.220	23.031	3.270	1.00	24.04
ATOM	541	O	ASP	255	26.032	23.249	3.080	1.00	23.66
ATOM	542	CB	ASP	255	28.706	25.025	3.334	1.00	30.25
ATOM	543	CG	ASP	255	29.970	25.689	2.881	1.00	35.18
ATOM	544	OD1	ASP	255	30.658	25.107	2.008	1.00	44.00
ATOM	545	OD2	ASP	255	30.295	26.799	3.367	1.00	43.40
ATOM	546	N	ASN	256	27.651	22.144	4.138	1.00	23.36
ATOM	547	CA	ASN	256	26.775	21.433	5.067	1.00	22.13
ATOM	548	C	ASN	256	25.746	20.545	4.364	1.00	20.90
ATOM	549	O	ASN	256	24.587	20.407	4.774	1.00	19.19
ATOM	550	CB	ASN	256	26.045	22.434	5.991	1.00	23.91
ATOM	551	CG	ASN	256	27.080	23.300	6.722	1.00	25.01
ATOM	552	OD1	ASN	256	28.095	22.760	7.143	1.00	27.40
ATOM	553	ND2	ASN	256	26.814	24.595	6.843	1.00	27.33
ATOM	554	N	LEU	257	26.203	19.945	3.284	1.00	20.22
ATOM	555	CA	LEU	257	25.373	19.175	2.390	1.00	18.95
ATOM	556	C	LEU	257	25.468	17.680	2.684	1.00	18.86
ATOM	557	O	LEU	257	26.592	17.123	2.645	1.00	21.67
ATOM	558	CB	LEU	257	25.795	19.404	0.922	1.00	19.51
ATOM	559	CG	LEU	257	24.846	18.848	-0.145	1.00	19.18
ATOM	560	CD1	LEU	257	23.496	19.559	-0.114	1.00	19.32
ATOM	561	CD2	LEU	257	25.480	18.963	-1.540	1.00	21.80
ATOM	562	N	PHE	258	24.343	17.030	2.952	1.00	18.87

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ATOM    563  CA  PHE   258      24.327  15.552   3.013  1.00 17.88
ATOM    564  C   PHE   258      23.464  15.028   1.872  1.00 17.15
ATOM    565  O   PHE   258      22.783  15.768   1.169  1.00 17.95
ATOM    566  CB  PHE   258      23.854  15.052   4.375  1.00 17.98
ATOM    567  CG  PHE   258      22.433  15.313   4.841  1.00 17.30
ATOM    568  CD1 PHE   258      22.070  16.544   5.406  1.00 17.90
ATOM    569  CD2 PHE   258      21.452  14.334   4.733  1.00 18.44
ATOM    570  CE1 PHE   258      20.755  16.766   5.832  1.00 17.83
ATOM    571  CE2 PHE   258      20.147  14.535   5.166  1.00 18.14
ATOM    572  CZ  PHE   258      19.796  15.753   5.734  1.00 18.79
ATOM    573  N   GLU   259      23.528  13.692   1.726  1.00 17.16
ATOM    574  CA  GLU   259      22.715  13.063   0.676  1.00 17.74
ATOM    575  C   GLU   259      22.000  11.852   1.230  1.00 17.08
ATOM    576  O   GLU   259      22.484  11.225   2.165  1.00 18.86
ATOM    577  CB  GLU   259      23.578  12.695  -0.533  1.00 20.20
ATOM    578  CG  GLU   259      24.723  11.719  -0.228  1.00 22.07
ATOM    579  CD  GLU   259      25.587  11.420  -1.440  1.00 23.90
ATOM    580  OE1 GLU   259      25.645  12.247  -2.395  1.00 28.98
ATOM    581  OE2 GLU   259      26.238  10.351  -1.441  1.00 29.10
ATOM    582  N   ILE   260      20.871  11.560   0.606  1.00 16.75
ATOM    583  CA  ILE   260      20.070  10.363   0.909  1.00 17.27
ATOM    584  C   ILE   260      19.979   9.581  -0.394  1.00 18.28
ATOM    585  O   ILE   260      19.362  10.064  -1.359  1.00 18.28
ATOM    586  CB  ILE   260      18.657  10.740   1.434  1.00 17.37
ATOM    587  CG1 ILE   260      18.696  11.694   2.630  1.00 16.97
ATOM    588  CG2 ILE   260      17.824   9.471   1.670  1.00 19.72
ATOM    589  CD1 ILE   260      17.411  12.374   3.022  1.00 20.30
ATOM    590  N   VAL   261      20.610   8.387  -0.415  1.00 18.12
ATOM    591  CA  VAL   261      20.703   7.645  -1.671  1.00 19.95
ATOM    592  C   VAL   261      19.611   6.603  -1.723  1.00 19.48
ATOM    593  O   VAL   261      19.571   5.736  -0.835  1.00 22.32
ATOM    594  CB  VAL   261      22.070   6.961  -1.767  1.00 20.79
ATOM    595  CG1 VAL   261      22.128   6.068  -2.997  1.00 25.37
ATOM    596  CG2 VAL   261      23.184   8.016  -1.738  1.00 24.27
ATOM    597  N   THR   262      18.755   6.662  -2.742  1.00 20.58
ATOM    598  CA  THR   262      17.790   5.588  -3.006  1.00 21.58
ATOM    599  C   THR   262      18.048   5.068  -4.423  1.00 22.67
ATOM    600  O   THR   262      18.792   5.698  -5.200  1.00 24.03
ATOM    601  CB  THR   262      16.322   6.016  -2.864  1.00 21.31
ATOM    602  OG1 THR   262      15.969   6.881  -3.968  1.00 21.88
ATOM    603  CG2 THR   262      16.043   6.822  -1.609  1.00 22.41
ATOM    604  N   THR   263      17.460   3.940  -4.822  1.00 24.28
ATOM    605  CA  THR   263      17.748   3.453  -6.168  1.00 27.65
ATOM    606  C   THR   263      17.372   4.470  -7.252  1.00 28.90
ATOM    607  O   THR   263      18.145   4.743  -8.176  1.00 32.23
ATOM    608  CB  THR   263      17.013   2.131  -6.406  1.00 29.19
ATOM    609  OG1 THR   263      17.554   1.150  -5.482  1.00 33.75
ATOM    610  CG2 THR   263      17.259   1.661  -7.824  1.00 32.03
ATOM    611  N   SER   264      16.190   5.062  -7.156  1.00 29.66
ATOM    612  CA  SER   264      15.729   5.914  -8.243  1.00 30.61
ATOM    613  C   SER   264      16.278   7.336  -8.184  1.00 28.06
ATOM    614  O   SER   264      16.265   8.029  -9.216  1.00 31.02
ATOM    615  CB  SER   264      14.196   6.039  -8.282  1.00 33.56
ATOM    616  OG  SER   264      13.664   6.525  -7.068  1.00 43.62
ATOM    617  N   ARG   265      16.729   7.762  -7.007  1.00 24.87
ATOM    618  CA  ARG   265      17.109   9.153  -6.839  1.00 23.38
ATOM    619  C   ARG   265      18.019   9.346  -5.635  1.00 21.19
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ATOM	620	O	ARG	265	17.821	8.800	-4.555	1.00	22.06
ATOM	621	CB	ARG	265	15.875	10.037	-6.632	1.00	24.21
ATOM	622	CG	ARG	265	16.215	11.533	-6.606	1.00	26.09
ATOM	623	CD	ARG	265	14.946	12.340	-6.810	1.00	26.56
ATOM	624	NE	ARG	265	15.204	13.759	-6.539	1.00	25.50
ATOM	625	CZ	ARG	265	14.240	14.673	-6.517	1.00	26.22
ATOM	626	NH1	ARG	265	12.970	14.349	-6.746	1.00	31.83
ATOM	627	NH2	ARG	265	14.557	15.926	-6.262	1.00	28.55
ATOM	628	N	THR	266	19.023	10.213	-5.821	1.00	21.49
ATOM	629	CA	THR	266	19.784	10.701	-4.671	1.00	20.67
ATOM	630	C	THR	266	19.265	12.089	-4.334	1.00	18.66
ATOM	631	O	THR	266	19.201	12.967	-5.209	1.00	21.71
ATOM	632	CB	THR	266	21.300	10.690	-4.942	1.00	22.24
ATOM	633	OG1	THR	266	21.683	9.294	-5.058	1.00	24.65
ATOM	634	CG2	THR	266	22.086	11.340	-3.814	1.00	21.58
ATOM	635	N	PHE	267	18.884	12.298	-3.091	1.00	17.12
ATOM	636	CA	PHE	267	18.431	13.595	-2.616	1.00	16.85
ATOM	637	C	PHE	267	19.590	14.369	-2.014	1.00	15.86
ATOM	638	O	PHE	267	20.347	13.768	-1.223	1.00	18.68
ATOM	639	CB	PHE	267	17.312	13.394	-1.577	1.00	16.99
ATOM	640	CG	PHE	267	16.098	12.727	-2.194	1.00	18.27
ATOM	641	CD1	PHE	267	15.085	13.491	-2.791	1.00	19.88
ATOM	642	CD2	PHE	267	15.982	11.319	-2.172	1.00	18.66
ATOM	643	CE1	PHE	267	13.963	12.861	-3.347	1.00	22.37
ATOM	644	CE2	PHE	267	14.854	10.726	-2.718	1.00	21.61
ATOM	645	CZ	PHE	267	13.870	11.488	-3.315	1.00	22.75
ATOM	646	N	TYR	268	19.676	15.655	-2.341	1.00	16.20
ATOM	647	CA	TYR	268	20.801	16.478	-1.858	1.00	16.28
ATOM	648	C	TYR	268	20.202	17.493	-0.894	1.00	15.18
ATOM	649	O	TYR	268	19.346	18.262	-1.318	1.00	16.38
ATOM	650	CB	TYR	268	21.539	17.172	-3.015	1.00	17.61
ATOM	651	CG	TYR	268	22.094	16.125	-3.964	1.00	18.68
ATOM	652	CD1	TYR	268	23.249	15.420	-3.665	1.00	21.29
ATOM	653	CD2	TYR	268	21.447	15.841	-5.174	1.00	20.52
ATOM	654	CE1	TYR	268	23.758	14.453	-4.544	1.00	23.26
ATOM	655	CE2	TYR	268	21.947	14.884	-6.032	1.00	22.13
ATOM	656	CZ	TYR	268	23.099	14.196	-5.717	1.00	23.72
ATOM	657	OH	TYR	268	23.600	13.228	-6.602	1.00	29.35
ATOM	658	N	VAL	269	20.616	17.443	0.360	1.00	15.34
ATOM	659	CA	VAL	269	19.940	18.154	1.445	1.00	14.73
ATOM	660	C	VAL	269	20.969	18.975	2.219	1.00	15.52
ATOM	661	O	VAL	269	21.893	18.442	2.828	1.00	16.49
ATOM	662	CB	VAL	269	19.189	17.167	2.360	1.00	15.60
ATOM	663	CG1	VAL	269	18.383	17.896	3.420	1.00	16.89
ATOM	664	CG2	VAL	269	18.305	16.246	1.525	1.00	17.16
ATOM	665	N	GLN	270	20.783	20.304	2.196	1.00	16.04
ATOM	666	CA	GLN	270	21.694	21.210	2.890	1.00	16.42
ATOM	667	C	GLN	270	21.125	21.605	4.240	1.00	16.16
ATOM	668	O	GLN	270	20.038	22.191	4.328	1.00	17.29
ATOM	669	CB	GLN	270	21.924	22.455	2.015	1.00	17.99
ATOM	670	CG	GLN	270	22.862	23.443	2.696	1.00	19.87
ATOM	671	CD	GLN	270	23.070	24.686	1.863	1.00	21.04
ATOM	672	OE1	GLN	270	22.134	25.235	1.278	1.00	22.53
ATOM	673	NE2	GLN	270	24.338	25.107	1.843	1.00	23.86
ATOM	674	N	ALA	271	21.830	21.253	5.311	1.00	16.14
ATOM	675	CA	ALA	271	21.451	21.680	6.657	1.00	16.59
ATOM	676	C	ALA	271	21.901	23.118	6.905	1.00	18.17

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ATOM	677	O	ALA	271	22.624	23.711	6.105	1.00	18.67
ATOM	678	CB	ALA	271	22.055	20.741	7.689	1.00	18.69
ATOM	679	N	ASP	272	21.436	23.669	8.040	1.00	18.64
ATOM	680	CA	ASP	272	21.672	25.093	8.294	1.00	19.44
ATOM	681	C	ASP	272	23.020	25.386	8.956	1.00	21.24
ATOM	682	O	ASP	272	23.350	26.541	9.240	1.00	22.29
ATOM	683	CB	ASP	272	20.541	25.672	9.137	1.00	19.84
ATOM	684	CG	ASP	272	20.480	25.240	10.577	1.00	20.07
ATOM	685	OD1	ASP	272	21.432	24.680	11.135	1.00	23.20
ATOM	686	OD2	ASP	272	19.403	25.493	11.187	1.00	23.28
ATOM	687	N	SER	273	23.789	24.342	9.200	1.00	21.09
ATOM	688	CA	SER	273	25.042	24.423	9.943	1.00	22.81
ATOM	689	C	SER	273	25.748	23.077	9.864	1.00	24.16
ATOM	690	O	SER	273	25.124	22.037	9.603	1.00	22.58
ATOM	691	CB	SER	273	24.799	24.781	11.422	1.00	23.57
ATOM	692	OG	SER	273	23.909	23.853	12.021	1.00	23.06
ATOM	693	N	PRO	274	27.043	23.053	10.125	1.00	26.63
ATOM	694	CA	PRO	274	27.692	21.754	10.213	1.00	27.87
ATOM	695	C	PRO	274	27.051	20.905	11.304	1.00	27.15
ATOM	696	O	PRO	274	26.911	19.690	11.108	1.00	27.01
ATOM	697	CB	PRO	274	29.137	22.062	10.631	1.00	29.54
ATOM	698	CG	PRO	274	29.286	23.537	10.592	1.00	30.09
ATOM	699	CD	PRO	274	27.953	24.179	10.340	1.00	28.30
ATOM	700	N	GLU	275	26.689	21.561	12.422	1.00	27.80
ATOM	701	CA	GLU	275	26.107	20.779	13.527	1.00	28.29
ATOM	702	C	GLU	275	24.821	20.088	13.102	1.00	25.41
ATOM	703	O	GLU	275	24.568	18.915	13.414	1.00	26.55
ATOM	704	CB	GLU	275	25.895	21.692	14.760	1.00	32.09
ATOM	705	CG	GLU	275	27.196	22.216	15.322	1.00	35.50
ATOM	706	CD	GLU	275	27.755	23.474	14.712	1.00	37.50
ATOM	707	OE1	GLU	275	27.232	23.962	13.705	1.00	34.89
ATOM	708	OE2	GLU	275	28.764	24.015	15.233	1.00	44.93
ATOM	709	N	GLU	276	23.972	20.827	12.372	1.00	22.31
ATOM	710	CA	GLU	276	22.721	20.195	11.941	1.00	20.22
ATOM	711	C	GLU	276	22.929	19.182	10.848	1.00	18.53
ATOM	712	O	GLU	276	22.149	18.223	10.771	1.00	19.40
ATOM	713	CB	GLU	276	21.715	21.291	11.522	1.00	18.69
ATOM	714	CG	GLU	276	21.059	21.942	12.757	1.00	20.67
ATOM	715	CD	GLU	276	20.420	20.909	13.672	1.00	22.48
ATOM	716	OE1	GLU	276	19.550	20.145	13.211	1.00	22.34
ATOM	717	OE2	GLU	276	20.753	20.880	14.873	1.00	26.46
ATOM	718	N	MET	277	23.956	19.346	10.002	1.00	20.24
ATOM	719	CA	MET	277	24.219	18.289	9.009	1.00	19.56
ATOM	720	C	MET	277	24.543	16.987	9.728	1.00	21.63
ATOM	721	O	MET	277	23.984	15.919	9.419	1.00	20.87
ATOM	722	CB	MET	277	25.356	18.721	8.063	1.00	21.09
ATOM	723	CG	MET	277	25.637	17.663	6.984	1.00	21.69
ATOM	724	SD	MET	277	27.226	17.847	6.137	1.00	25.27
ATOM	725	CE	MET	277	28.378	17.423	7.427	1.00	33.19
ATOM	726	N	HIS	278	25.457	17.043	10.709	1.00	24.84
ATOM	727	CA	HIS	278	25.789	15.823	11.468	1.00	26.09
ATOM	728	C	HIS	278	24.582	15.291	12.223	1.00	24.71
ATOM	729	O	HIS	278	24.372	14.066	12.299	1.00	26.36
ATOM	730	CB	HIS	278	26.997	16.132	12.374	1.00	29.33
ATOM	731	CG	HIS	278	28.242	16.390	11.561	1.00	31.94
ATOM	732	ND1	HIS	278	28.807	15.424	10.755	1.00	34.30
ATOM	733	CD2	HIS	278	29.010	17.499	11.410	1.00	33.93

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ATOM	734	CE1	HIS	278	29.883	15.922	10.151	1.00 35.12
ATOM	735	NE2	HIS	278	30.032	17.188	10.531	1.00 35.31
ATOM	736	N	SER	279	23.749	16.189	12.780	1.00 24.49
ATOM	737	CA	SER	279	22.568	15.731	13.535	1.00 23.54
ATOM	738	C	SER	279	21.618	14.949	12.633	1.00 21.01
ATOM	739	O	SER	279	21.077	13.906	13.015	1.00 22.61
ATOM	740	CB	SER	279	21.874	16.930	14.190	1.00 23.48
ATOM	741	OG	SER	279	20.749	16.532	14.951	1.00 24.61
ATOM	742	N	TRP	280	21.387	15.470	11.423	1.00 18.60
ATOM	743	CA	TRP	280	20.488	14.785	10.492	1.00 17.77
ATOM	744	C	TRP	280	21.063	13.443	10.079	1.00 18.71
ATOM	745	O	TRP	280	20.350	12.438	10.081	1.00 19.23
ATOM	746	CB	TRP	280	20.220	15.610	9.215	1.00 17.19
ATOM	747	CG	TRP	280	19.123	16.620	9.424	1.00 15.75
ATOM	748	CD1	TRP	280	19.227	17.997	9.590	1.00 16.48
ATOM	749	CD2	TRP	280	17.723	16.296	9.496	1.00 15.68
ATOM	750	NE1	TRP	280	17.965	18.542	9.753	1.00 16.21
ATOM	751	CE2	TRP	280	17.041	17.502	9.703	1.00 15.55
ATOM	752	CE3	TRP	280	17.000	15.099	9.412	1.00 16.18
ATOM	753	CZ2	TRP	280	15.658	17.562	9.824	1.00 16.08
ATOM	754	CZ3	TRP	280	15.618	15.138	9.530	1.00 16.55
ATOM	755	CH2	TRP	280	14.961	16.370	9.735	1.00 16.86
ATOM	756	N	ILE	281	22.340	13.418	9.708	1.00 20.02
ATOM	757	CA	ILE	281	22.935	12.132	9.291	1.00 21.24
ATOM	758	C	ILE	281	22.848	11.120	10.432	1.00 22.54
ATOM	759	O	ILE	281	22.493	9.946	10.235	1.00 23.66
ATOM	760	CB	ILE	281	24.382	12.288	8.824	1.00 21.19
ATOM	761	CG1	ILE	281	24.481	13.137	7.538	1.00 20.98
ATOM	762	CG2	ILE	281	25.059	10.933	8.650	1.00 23.71
ATOM	763	CD1	ILE	281	25.909	13.643	7.351	1.00 22.54
ATOM	764	N	LYS	282	23.172	11.567	11.647	1.00 22.97
ATOM	765	CA	LYS	282	23.150	10.624	12.763	1.00 24.74
ATOM	766	C	LYS	282	21.743	10.126	13.074	1.00 23.46
ATOM	767	O	LYS	282	21.521	8.956	13.392	1.00 24.20
ATOM	768	CB	LYS	282	23.745	11.328	13.988	1.00 27.48
ATOM	769	CG	LYS	282	24.001	10.410	15.169	1.00 29.66
ATOM	770	CD	LYS	282	24.462	11.238	16.364	1.00 32.91
ATOM	771	CE	LYS	282	24.165	10.520	17.671	0.00 32.72
ATOM	772	NZ	LYS	282	24.633	11.299	18.851	0.00 32.90
ATOM	773	N	ALA	283	20.749	11.017	13.005	1.00 23.29
ATOM	774	CA	ALA	283	19.388	10.630	13.314	1.00 22.18
ATOM	775	C	ALA	283	18.838	9.667	12.267	1.00 20.90
ATOM	776	O	ALA	283	18.210	8.667	12.600	1.00 23.15
ATOM	777	CB	ALA	283	18.475	11.867	13.427	1.00 22.41
ATOM	778	N	VAL	284	19.052	9.955	10.994	1.00 20.29
ATOM	779	CA	VAL	284	18.518	9.042	9.962	1.00 19.70
ATOM	780	C	VAL	284	19.217	7.687	10.042	1.00 22.54
ATOM	781	O	VAL	284	18.576	6.634	9.953	1.00 24.00
ATOM	782	CB	VAL	284	18.636	9.651	8.559	1.00 19.96
ATOM	783	CG1	VAL	284	18.124	8.649	7.516	1.00 23.69
ATOM	784	CG2	VAL	284	17.847	10.951	8.446	1.00 19.56
ATOM	785	N	SER	285	20.543	7.718	10.208	1.00 23.36
ATOM	786	CA	SER	285	21.270	6.464	10.296	1.00 25.37
ATOM	787	C	SER	285	20.854	5.670	11.521	1.00 27.45
ATOM	788	O	SER	285	20.772	4.440	11.461	1.00 30.18
ATOM	789	CB	SER	285	22.770	6.739	10.224	0.50 26.62
ATOM	790	OG	SER	285	23.188	7.490	11.333	0.50 31.87

Figure 9: page 14

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ATOM    791  N   GLY   286      20.573   6.322  12.659  1.00 28.00
ATOM    792  CA  GLY   286      20.140   5.546  13.821  1.00 29.85
ATOM    793  C   GLY   286      18.768   4.932  13.600  1.00 29.16
ATOM    794  O   GLY   286      18.468   3.812  14.030  1.00 32.59
ATOM    795  N   ALA   287      17.897   5.671  12.909  1.00 27.31
ATOM    796  CA  ALA   287      16.586   5.074  12.665  1.00 26.79
ATOM    797  C   ALA   287      16.694   3.862  11.742  1.00 27.29
ATOM    798  O   ALA   287      15.976   2.888  11.880  1.00 30.26
ATOM    799  CB  ALA   287      15.677   6.171  12.124  1.00 27.49
ATOM    800  N   ILE   288      17.584   3.894  10.761  1.00 27.62
ATOM    801  CA  ILE   288      17.784   2.738   9.885  1.00 29.23
ATOM    802  C   ILE   288      18.334   1.578  10.676  1.00 32.35
ATOM    803  O   ILE   288      17.876   0.442  10.509  1.00 34.51
ATOM    804  CB  ILE   288      18.735   3.137   8.744  1.00 27.90
ATOM    805  CG1 ILE   288      18.024   4.088   7.765  1.00 26.55
ATOM    806  CG2 ILE   288      19.342   1.937   8.045  1.00 32.92
ATOM    807  CD1 ILE   288      19.008   4.733   6.800  1.00 24.28
ATOM    808  N   VAL   289      19.308   1.848  11.548  1.00 33.56
ATOM    809  CA  VAL   289      19.832   0.777  12.410  1.00 36.04
ATOM    810  C   VAL   289      18.705   0.142  13.219  1.00 35.53
ATOM    811  O   VAL   289      18.579  -1.074  13.365  1.00 36.75
ATOM    812  CB  VAL   289      20.919   1.282  13.367  1.00 38.07
ATOM    813  CG1 VAL   289      21.273   0.194  14.374  1.00 41.96
ATOM    814  CG2 VAL   289      22.178   1.716  12.630  1.00 40.34
ATOM    815  N   ALA   290      17.856   0.989  13.801  1.00 35.20
ATOM    816  CA  ALA   290      16.787   0.417  14.617  1.00 36.68
ATOM    817  C   ALA   290      15.877  -0.496  13.800  1.00 38.50
ATOM    818  O   ALA   290      15.181  -1.346  14.362  1.00 43.70
ATOM    819  CB  ALA   290      15.957   1.516  15.261  1.00 36.03
ATOM    820  N   GLN   291      15.819  -0.337  12.481  1.00 38.88
ATOM    821  CA  GLN   291      15.007  -1.231  11.674  1.00 39.19
ATOM    822  C   GLN   291      15.638  -2.607  11.471  1.00 40.84
ATOM    823  O   GLN   291      14.985  -3.525  10.958  1.00 43.89
ATOM    824  CB  GLN   291      14.765  -0.645  10.273  1.00 37.40
ATOM    825  CG  GLN   291      13.734   0.465  10.210  1.00 37.77
ATOM    826  CD  GLN   291      13.397   0.925   8.803  1.00 37.69
ATOM    827  OE1 GLN   291      14.259   0.949   7.915  1.00 42.16
ATOM    828  NE2 GLN   291      12.141   1.321   8.598  1.00 33.52
ATOM    829  N   ARG   292      16.908  -2.762  11.831  1.00 43.40
ATOM    830  CA  ARG   292      17.576  -4.019  11.498  1.00 46.96
ATOM    831  C   ARG   292      17.017  -5.214  12.257  1.00 48.67
ATOM    832  O'  ARG   292      16.450  -5.062  13.356  1.00 51.24
ATOM    833  CB  ARG   292      19.090  -3.871  11.752  1.00 49.04
ATOM    834  CG  ARG   292      19.740  -3.015  10.690  1.00 51.73
ATOM    835  CD  ARG   292      21.167  -2.561  10.976  1.00 54.98
ATOM    836  NE  ARG   292      21.464  -1.454  10.064  1.00 57.80
ATOM    837  CZ  ARG   292      22.320  -1.371   9.067  1.00 59.63
ATOM    838  NH1 ARG   292      23.113  -2.388   8.751  1.00 66.18
ATOM    839  NH2 ARG   292      22.406  -0.239   8.364  1.00 59.69
ATOM    840  O'' ARG   292      17.165  -6.320  11.693  1.00 51.17
TER
HETATM  841  O1  CIT  2001       9.981  20.451  -2.421  1.00 65.34
HETATM  842  O2  CIT  2001       9.194  21.475  -4.223  1.00 68.02
HETATM  843  O3  CIT  2001       9.507  15.110  -5.235  1.00 68.53
HETATM  844  O4  CIT  2001       7.822  16.537  -4.989  1.00 66.82
HETATM  845  O5  CIT  2001      10.625  18.666  -7.683  1.00 62.45
HETATM  846  O6  CIT  2001      12.275  17.856  -6.394  1.00 41.55
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Figure 9: page 15

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HETATM  847  O7  CIT  2001       8.923  19.051  -5.829  1.00 68.64
HETATM  848  C1  CIT  2001       9.939  20.616  -3.650  1.00 66.56
HETATM  849  C2  CIT  2001      10.831  19.687  -4.460  1.00 65.28
HETATM  850  C3  CIT  2001      10.209  18.597  -5.358  1.00 63.38
HETATM  851  C4  CIT  2001      10.044  17.321  -4.527  1.00 64.72
HETATM  852  C5  CIT  2001       9.055  16.247  -4.950  1.00 66.10
HETATM  853  C6  CIT  2001      11.124  18.347  -6.576  1.00 59.88
TER
HETATM  854  O   HOH  3001      19.039  22.441   9.037  1.00 18.13
HETATM  855  O   HOH  3002      24.421  25.821   5.756  1.00 28.70
HETATM  856  O   HOH  3003      17.936  21.106  11.246  1.00 19.30
HETATM  857  O   HOH  3004      17.508  16.551  -4.023  1.00 20.51
HETATM  858  O   HOH  3005      15.811   2.568  -2.991  1.00 24.54
HETATM  859  O   HOH  3006      14.311  23.262  -9.680  1.00 22.11
HETATM  860  O   HOH  3007      10.794   2.031   6.023  1.00 26.75
HETATM  861  O   HOH  3008      17.809  14.933  -6.207  1.00 26.49
HETATM  862  O   HOH  3009      15.853  19.509  14.582  1.00 25.19
HETATM  863  O   HOH  3010       3.741  20.018   1.757  1.00 25.10
HETATM  864  O   HOH  3011      20.360  27.415  -8.342  1.00 26.25
HETATM  865  O   HOH  3012      13.566   3.159  13.110  1.00 29.47
HETATM  866  O   HOH  3013      14.581  27.939  -5.843  1.00 28.13
HETATM  867  O   HOH  3014      28.793  10.981   6.612  1.00 37.13
HETATM  868  O   HOH  3015      13.523  17.942  16.006  1.00 26.00
HETATM  869  O   HOH  3016      17.446   8.159  15.286  1.00 30.76
HETATM  870  O   HOH  3017      24.404  23.455  -7.043  1.00 30.46
HETATM  871  O   HOH  3018       6.022  10.249  -1.870  1.00 32.45
HETATM  872  O   HOH  3019      20.496   7.282  -6.618  1.00 29.50
HETATM  873  O   HOH  3020      24.292   9.010  -5.584  1.00 34.54
HETATM  874  O   HOH  3021      -4.725  20.205  13.015  1.00 34.60
HETATM  875  O   HOH  3022      17.860  16.637  16.811  1.00 37.96
HETATM  876  O   HOH  3023      22.756   6.729  14.696  1.00 50.38
HETATM  877  O   HOH  3024      25.726  17.678  15.668  1.00 37.17
HETATM  878  O   HOH  3025      26.569   8.844  -3.503  1.00 33.61
HETATM  879  O   HOH  3026      22.448  22.267  16.358  1.00 35.70
HETATM  880  O   HOH  3027      21.342  27.738   2.078  1.00 46.00
HETATM  881  O   HOH  3028      29.017  18.818   2.673  1.00 33.85
HETATM  882  O   HOH  3029       7.289  18.242  13.091  1.00 42.49
HETATM  883  O   HOH  3030      22.036  12.304  -8.712  1.00 41.31
HETATM  884  O   HOH  3031      20.630   1.544   4.852  1.00 41.53
HETATM  885  O   HOH  3032       2.109  21.329  -0.211  1.00 39.26
HETATM  886  O   HOH  3033      15.703  22.178 -17.009  1.00 38.88
HETATM  887  O   HOH  3034      23.417   0.033   1.940  1.00 42.13
HETATM  888  O   HOH  3035       9.299  -0.393  -1.833  1.00 44.89
HETATM  889  O   HOH  3036      12.541   0.766  14.088  1.00 42.88
HETATM  890  O   HOH  3037      30.053  20.193  13.422  1.00 43.81
HETATM  891  O   HOH  3038      11.793   6.532  -2.007  1.00 40.86
HETATM  892  O   HOH  3039      16.197  21.930  13.272  1.00 22.05
HETATM  893  O   HOH  3040      18.479  27.852  -6.327  1.00 22.97
HETATM  894  O   HOH  3041      20.925  13.276  15.805  1.00 28.82
HETATM  895  O   HOH  3042      22.084  25.561  -8.281  1.00 29.61
HETATM  896  O   HOH  3043      19.496  11.268  -8.436  1.00 32.74
HETATM  897  O   HOH  3044      24.545   5.224   5.218  1.00 31.84
HETATM  898  O   HOH  3045      15.970   6.108  16.216  1.00 35.96
HETATM  899  O   HOH  3046      26.873  27.086   0.981  1.00 34.36
HETATM  900  O   HOH  3047      13.731   4.474  15.369  1.00 36.93
HETATM  901  O   HOH  3048      17.662  10.094  16.627  1.00 43.45
HETATM  902  O   HOH  3049      11.843  20.255  15.575  1.00 37.43
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Figure 9: page 16

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HETATM  903   O   HOH  3050      22.343  28.043  11.486  1.00 32.88
HETATM  904   O   HOH  3051      21.980  23.263  -9.008  1.00 33.42
HETATM  905   O   HOH  3052      33.774  18.471  -4.454  1.00 54.47
HETATM  906   O   HOH  3053      25.260  14.836  15.796  1.00 40.17
HETATM  907   O   HOH  3054      30.626  21.743   4.386  1.00 38.17
HETATM  908   O   HOH  3055      24.620  27.856   0.504  1.00 39.32
HETATM  909   O   HOH  3056       7.725  25.507  15.979  1.00 51.08
HETATM  910   O   HOH  3057      28.305  17.702  15.630  1.00 41.93
HETATM  911   O   HOH  3058      16.911  -1.163  -6.463  1.00 38.51
HETATM  912   O   HOH  3059      27.947  12.828  10.356  1.00 44.34
HETATM  913   O   HOH  3060       3.192  14.753  10.116  1.00 45.99
HETATM  914   O   HOH  3061      29.105  11.423  -1.839  1.00 34.73
HETATM  915   O   HOH  3062      20.621  10.756  16.791  1.00 33.10
HETATM  916   O   HOH  3063      27.067   8.207   7.150  1.00 35.93
HETATM  917   O   HOH  3064      13.270  28.316  -0.718  1.00 35.07
HETATM  918   O   HOH  3065      19.441   3.079  16.500  1.00 37.14
HETATM  919   O   HOH  3066      20.012  18.919  16.653  1.00 43.37
HETATM  920   O   HOH  3067       4.330  15.900  -4.251  1.00 43.75
HETATM  921   O   HOH  3068      22.597  17.451  -9.396  1.00 39.34
HETATM  922   O   HOH  3069      19.740  28.634  -0.916  1.00 37.99
HETATM  923   O   HOH  3070      -2.262  21.731   5.181  1.00 42.31
HETATM  924   O   HOH  3071      31.539  10.512   5.259  1.00 55.85
HETATM  925   O   HOH  3072      21.834  -3.258   5.427  1.00 38.47
HETATM  926   O   HOH  3073      17.963   4.781  17.562  1.00 44.37
HETATM  927   O   HOH  3074      23.066  14.427  17.246  1.00 36.86
HETATM  928   O   HOH  3075      22.468   5.435  -6.959  1.00 48.81
HETATM  929   O   HOH  3076      26.860  12.083  -4.381  1.00 44.29
HETATM  930   O   HOH  3077      30.566  10.902   2.235  1.00 43.04
HETATM  931   O   HOH  3078      23.639  -2.317   3.267  1.00 46.71
HETATM  932   O   HOH  3079      15.435  16.206  17.396  1.00 52.47
HETATM  933   O   HOH  3080      28.838   6.479   6.439  1.00 65.19
HETATM  934   O   HOH  3081      18.549   0.500  17.607  1.00 44.19
HETATM  935   O   HOH  3082      14.301  30.604   5.232  1.00 47.45
HETATM  936   O   HOH  3083      30.778  24.118   6.782  1.00 47.38
HETATM  937   O   HOH  3084      22.345   3.377  16.545  1.00 46.42
HETATM  938   O   HOH  3085       8.085  11.357   8.225  1.00 40.46
HETATM  939   O   HOH  3086      13.101  22.368  16.835  1.00 31.36
HETATM  940   O   HOH  3087      32.595  23.787   8.135  1.00 45.82
HETATM  941   O   HOH  3088       9.241   5.059  -2.552  1.00 61.15
HETATM  942   O   HOH  3089      15.580  -7.823  12.581  1.00 67.27
HETATM  943   O   HOH  3090      11.835   9.266  -5.592  1.00 41.12
HETATM  944   O   HOH  3091      11.210  17.266  16.453  1.00 62.30
HETATM  945   O   HOH  3092       7.704  27.341  10.085  1.00 31.66
HETATM  946   O   HOH  3093      30.618   9.227  -1.439  1.00 48.61
HETATM  947   O   HOH  3094       6.594  25.975  -3.429  1.00 54.30
HETATM  948   O   HOH  3095      18.630  16.770  -8.010  1.00 41.92
HETATM  949   O   HOH  3096      32.508  25.285  10.368  0.50 45.24
HETATM  950   O   HOH  3097      28.422  28.457   5.028  1.00 51.25
HETATM  951   O   HOH  3098       6.499  12.129   6.767  1.00 54.35
HETATM  952   O   HOH  3099      33.173  17.515  -1.409  1.00 51.87
HETATM  953   O   HOH  3100      16.484  13.627  15.948  1.00 75.57
HETATM  954   O   HOH  3101       7.586   1.698  -0.786  1.00 58.09
HETATM  955   O   HOH  3102       9.377  29.445   8.591  1.00 48.75
HETATM  956   O   HOH  3103      11.968   0.910  16.288  1.00 85.99
HETATM  957   O   HOH  3104      19.571  15.017  -9.161  1.00 59.44
HETATM  958   O   HOH  3105      19.903  30.791   4.913  1.00 79.58
HETATM  959   O   HOH  3106      24.302  13.649 -10.220  1.00 60.27
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Figure 9: page 17

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HETATM  960   O    HOH  3107       8.478  -0.899   8.733  1.00 78.45
HETATM  961   O    HOH  3108      12.204  21.654  -8.737  1.00 36.17
HETATM  962   O    HOH  3109      13.254   7.508  -4.127  1.00 40.71
HETATM  963   O    HOH  3110      20.530  -1.084   6.203  1.00 51.07
HETATM  964   O    HOH  3111      13.894  21.976  -4.347  1.00 38.32
HETATM  965   O    HOH  3112       9.580   8.084  -2.351  1.00 44.06
HETATM  966   O    HOH  3113      10.563  21.596  14.059  1.00 50.57
HETATM  967   O    HOH  3114      13.037   2.862  17.718  1.00 52.41
HETATM  968   O    HOH  3115      -0.863  20.133   2.945  1.00 47.05
HETATM  969   O    HOH  3116      28.633  26.509   8.053  1.00 45.62
HETATM  970   O    HOH  3117      23.057  24.641  14.628  1.00 50.18
HETATM  971   O    HOH  3118      25.138   6.043   7.732  1.00 52.93
HETATM  972   O    HOH  3119      18.724  14.178  16.931  1.00 62.99
HETATM  973   O    HOH  3120      11.758   5.100  -4.836  1.00 56.78
HETATM  974   O    HOH  3121      16.184  20.297  17.260  1.00 53.57
HETATM  975   O    HOH  3122      28.411  25.925  13.534  1.00 70.15
HETATM  976   O    HOH  3123      20.295  31.366   2.729  1.00 67.00
HETATM  977   O    HOH  3124      26.235   8.792  11.779  1.00 49.94
HETATM  978   O    HOH  3125      14.803   7.190  18.217  1.00 52.72
HETATM  979   O    HOH  3126      29.636  20.890   7.366  1.00 75.48
HETATM  980   O    HOH  3127      21.388  16.621  17.456  1.00 57.55
HETATM  981   O    HOH  3128      13.774  12.447  19.072  1.00 56.39
HETATM  982   O    HOH  3129      25.294  28.335  -2.936  1.00 50.07
HETATM  983   O    HOH  3130      19.937  -7.040  10.081  1.00 68.47
HETATM  984   O    HOH  3131       7.685  28.705   5.575  1.00 71.83
HETATM  985   O    HOH  3132       5.325  12.727  16.832  1.00 64.95
HETATM  986   O    HOH  3133      13.825   9.748  -9.820  1.00 54.49
HETATM  987   O    HOH  3134      22.671  28.524   4.158  1.00 69.49
HETATM  988   O    HOH  3135      27.446  12.924  14.759  1.00 57.60
TER
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Figure 9: page 18

INTERNATIONAL SEARCH REPORT

Internat pplication No
PCT/GB 02/03262

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K14/47

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, WPI Data, EMBASE, SEQUENCE SEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FERGUSON KATHRYN M ET AL: "Structural basis for discrimination of 3-phosphoinositides by pleckstrin homology domains." MOLECULAR CELL, vol. 6, no. 2, August 2000 (2000-08), pages 373-384, XP002226095 ISSN: 1097-2765	9-12
Y	the whole document	1-12, 17-42, 50-55

-/--

[X] *Further documents are listed in the continuation of box C.*

[ ] *Patent family members are listed in annex.*

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 December 2002	15/01/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Wimmer, G

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	DOWLER S ET AL: "IDENTIFICATION OF PLECKSTRIN-HOMOLOGY-DOMAIN-CONTAINING PROTEINS WITH NOVEL PHOSPHOINOSITIDE-BINDING SPECIFICITIES" BIOCHEMICAL JOURNAL, THE BIOCHEMICAL SOCIETY, LONDON, GB, vol. 351, 2000, pages 19-31, XP001096514 ISSN: 0264-6021 the whole document ---	1-12, 17-42, 50-55
A	LIETZKE SUSAN E ET AL: "Structural basis of 3-phosphoinositide recognition by pleckstrin homology domains." MOLECULAR CELL, vol. 6, no. 2, August 2000 (2000-08), pages 385-394, XP002226098 ISSN: 1097-2765 the whole document ---	1-42, 50-55
A	LEEVERS SALLY J ET AL: "Signalling through phosphoinositide 3-kinases: The lipids take centre stage." CURRENT OPINION IN CELL BIOLOGY, vol. 11, no. 2, April 1999 (1999-04), pages 219-225, XP002226099 ISSN: 0955-0674 the whole document ---	1-42, 50-55
T	THOMAS C C ET AL: "CRYSTAL STRUCTURE OF THE PHOSPHATIDYLINOSITOL 3,4-BISPHOPHATE-BINDING PLECKSTRIN HOMOLOGY (PH) DOMAIN OF TANDEM PH-DOMAIN-CONTAINING PROTEIN 1 (TAPP1): MOLECULAR BASIS OF LIQUID SPECIFICITY" BIOCHEMICAL JOURNAL, THE BIOCHEMICAL SOCIETY, LONDON, GB, vol. 358, no. PART 2, 1 September 2001 (2001-09-01), pages 287-294, XP001057248 ISSN: 0264-6021 the whole document -----	1-42, 50-55

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 43-49

Present claims 43-49 relate to a compound defined by reference to a desirable characteristic or property, namely that it may be identified by a method according to claims 1-27. The claims cover all compounds having this characteristic, whereas the application provides support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT for only a very limited number of such compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the compound by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, a search could only be carried out for compounds which are sufficiently clearly defined and supported by the description, namely a citrate, PtdIns(3,4)P2 or PtdIns(3,4,5); however these compounds are disclaimed in claims 43-49. Consequently, a search for these claims could not be carried out.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 02/03262

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 43-49
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.